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ORIGINAL ARTICLES

BACTERIOLOGY AND PATHOLOGY IN SIX CASES OF ENCEPHALITIS LETHARGICA*

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THIS report is based on material from six cases of "Lethargic Encephalitis" coming to autopsy within seven days of one another. They represent the fatal portion of a larger series of cases which will be reported from the clinical standpoint by Freund, Lockwood, and Crump.

BACTERIOLOGIC PART

Isolation and Growth Characteristics of Organism.—Cultures were obtained at autopsy using the following technic. The brain was removed from the cranium and placed, vertex upward, on a dissecting board. The posterior horns of the lateral ventricles were opened under aseptic precautions by an incision through the after part of the cerebrum. Fluid was aspirated with a sterile pipette and planted on bouillon and several loops of material were taken with a platinum loop and planted on agar, blood serum, human blood agar, Kligler's medium and gelatin.

In 24 hours the bouillon was very cloudy, showing a diffuse growth and some precipitate, but no evidence of stalactite or pellicle formation. There was practically no odor to the culture. The colonies on the agar slants were small and discrete with well-defined borders slightly raised from the surface, glistening and yellowish white in color.

On Kligler's lead acetate modification of Russel's triple sugar medium the colonies were similar in morphology, faintly pink in color, with diffuse reddening of the medium. No gas and no browning of stab was noted.

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On blood serum the colonies were similar, but showed some digestion of the surrounding medium.

The glucose agar stab grew well throughout its length and was villous in character, without gas formation.

On human blood agar the growth is at first slight as noted by a few isolated colonies surrounded by a brown zone. Some of the primary cultures on human blood agar showed no growth at all, but subcultures grow well.

Gelatin stab cultures incubated at room temperature for 24 hours show finely villous growth throughout length of stab without liquefaction.

Transplants were made in xylose, saccharose, lactose, dextrose, salicin, mannite and plain bouillon with fermentation tubes and brom-cresol-purple as indicator. Fermentation took place in tubes containing lactose and dextrose with acid formation in twenty-four hours. There was slow and feeble fermentation with acid formation in saccharose. No gas formation took place in any of the tubes. The bouillon culture is not soluble in bile.

The growth, when removed from an agar slant, is mucoid in character, spreads freely on dry glass, has a tendency to agglutinate spontaneously when mixed with saline, and is then difficult to break up.

The organism is a nonmotile coccus, small in young cultures, as large as a staphylococcus in old cultures, with a tendency to grow in diplo and tetrad forms and to bunch in small clusters. It divides similarly to a staphylococcus in three planes, stains readily with the aniline dyes, and is Gram-positive.

Pure cultures were obtained from six consecutive autopsies, the organism found being identical in each case.

Agglutination.—Sera were obtained from two convalescent and three cases still at the height of the disease.

The two convalescent cases showed partial but incomplete agglutination in dilutions up to 1:40, but the agglutination was not complete in any dilution.

Of the three sick cases, one, who is still sick and will probably die, agglutinates the organism completely at 1:50 and partially at 1:100. The other two sick cases showed partial agglutination in dilutions of one to ten.

Control experiments with the serum of normal persons did not show agglutination.

ANIMAL EXPERIMENTS

EXPERIMENT I.—*To determine pathogenicity of the organism for rabbits.* A full grown rabbit was injected subdurally with 1 c.c. of a 24-hour bouillon culture under general anesthesia and aseptic precautions. Recovery from the operative procedure was prompt, and the animal was apparently well six hours later. The next morning the head was extended on the spine, the hind legs flaccid, and the forelegs showed continuous tremors. The respiration was slow but regular and rhythmical. It was very restless, shifting and throwing itself about the cage in an aimless manner. Active stimulation, such as a loud noise or jar, partially aroused the animal and caused it to make incoordinate movements with forelegs and head. These symptoms continued for two days, after which time it became quieter, and the respirations slower and more shallow. These symptoms progressed until its death, six days after injection. The autopsy showed no exudate on the meninges or base of the brain. The findings were similar to the findings observed in the human cases. The brain cortex was cherry red and there was passive congestion of the vessels of the pia and cerebrum. Cultures were taken of the posterior horns of the ventricles by searing the sur-

Fig. 1.—Perivascular infiltration. Blood vessel in the upper portion of the pons.

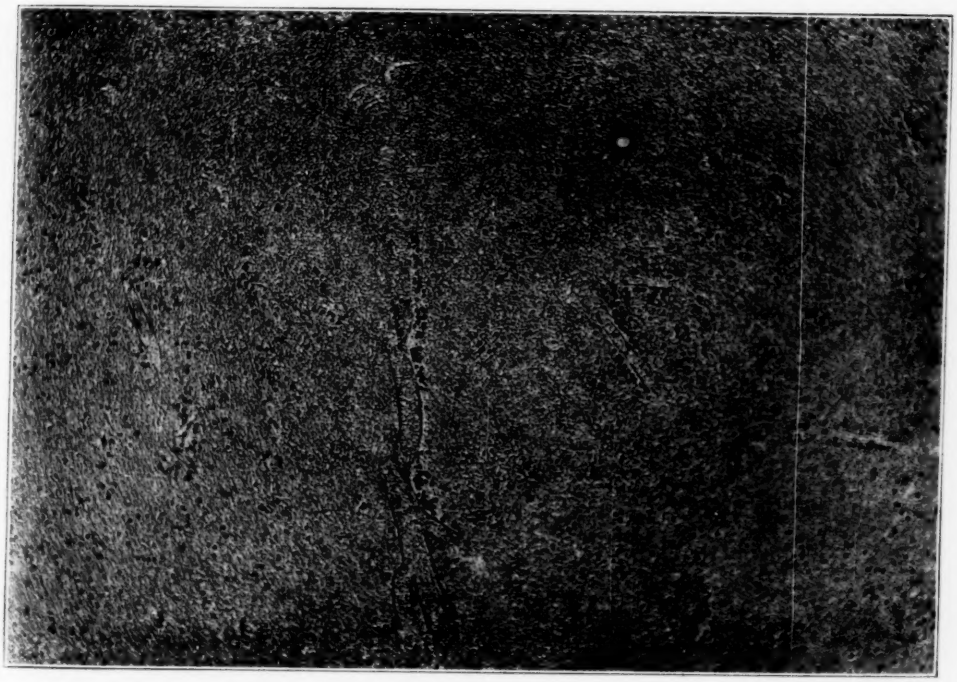

Fig. 2.—Small arterioles of the centrum semiovale showing perivascular collections of round cells.

face with a hot spatula and plunging a platinum loop into the brain. The organism was recovered in pure culture and found to be identical with the one injected.

EXPERIMENT II.—*To determine whether the organism was pathogenic by the intravenous route.* A full grown rabbit received 2 c.c. intravenously of the same culture as was used for Experiment I. No effects were noted, and at present, twelve days later, the animal is normal.

EXPERIMENT III.—*To determine the pathogenicity of the filtered culture.* The bouillon culture used for experiments one and two was allowed to grow five days. It was then filtered through a water-dammed Berkefeld "N" filter with a negative pressure of one-half atmosphere. One c.c. of this filtrate was used for subdural injection. The rest of the filtrate was incubated and also subcultures from it were incubated to determine its sterility. Both the original filtrate and the subcultures on all media including bouillon are still sterile. One c.c. of the five-day bouillon culture filtrate was injected subdurally in a full grown rabbit. There was immediate primary recovery from the operation, which was done about 6 P. M. The following morning the rabbit was in the typical lethargic state with symptoms exactly similar to those of the animal in Experiment I. This animal lived four days, and at autopsy showed intense injection of the meninges, brain edema, but no purulent exudate. Cultures were made by the same technic as in Experiment I and were all sterile. Intravenous injection of three c.c. of the bouillon culture filtrate in another animal produced no effect, and one c.c. intraperitoneally in a guinea pig was without result.

This result in Experiment III raised the question as to whether the pathologic effect was due to a toxin generated by the organism or to a filterable virus, as Loewe and Strauss[5] believe, or to the bouillon itself. Certain manifestations of the disease clinically, as well as the fact that a purulent exudate was never found, either in the human autopsies or in the animal experiments, strongly suggested the toxin or filterable virus idea. It was evident that if a filterable virus was present it grew well in bouillon, therefore, the bouillon filtrate transplants in bouillon could be used to determine this point. The following experiments were therefore carried out.

EXPERIMENT IV.—*Bouillon control.* Two c.c. of sterile bouillon of the same batch as that so far used for cultures was injected subdurally. No effects were noted. The animal remained entirely normal.

EXPERIMENT V.—*Injection of the bouillon subcultures from Berkefeld filtrate.* Two c.c. of the bouillon subcultures from the primary toxic filtrate were injected subdurally as in the previous experiments. No effects resulted, and the rabbit remained well. As before noted, these cultures were morphologically sterile and the toxic effect of the primary culture was removed by the dilution incident to subculturing.

Of course, another possibility remains that a filterable virus might still be present which would not grow except in symbiosis with the other organism and also that the organism itself might have a filterable stage in its development with unknown conditions for propagation. We leave this question as speculative and not subject to experimental elucidation.

Four attempts were made to grow the organism directly from the spinal fluid obtained at lumbar puncture without a success. One c.c. of spinal fluid from a sick case injected subdurally in a rabbit was without result. The search for the organism in smears of the sedimented spinal fluid has been negative. In smears from the ventricular fluid at autopsy there were found coccus-like bodies which could not be definitely diagnosed as the organism. In this connection it should

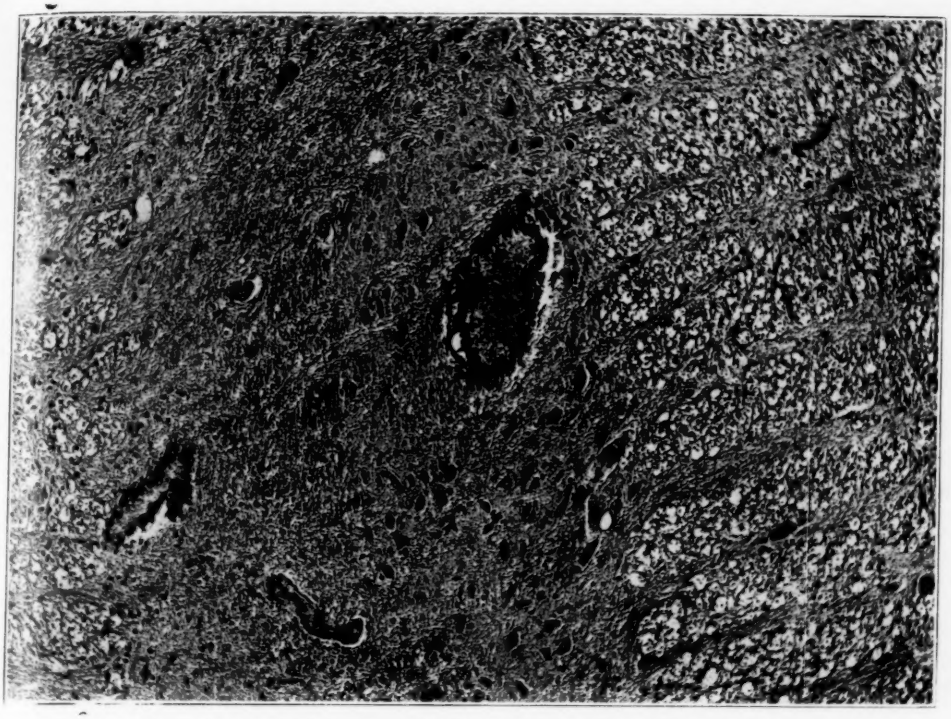

Fig. 3.—Vessel of the formatio reticularis in the region of the nucleus ambiguus.

Fig. 4.—Thalamic region. Unusually intense lesion with multiple miliary hemorrhages and intense perivascular infiltration.

be remembered that the localization of the disease seems to be entirely cerebral and in the brain stem, and as the pathologic study shows, especially of the white matter of the basal ganglia and pons. To get the cultures from the posterior horns, several loopfuls were planted on each tube or several drops of spinal fluid from a sterile pipette dropped into bouillon. Even then in one of our cases only one colony was found on two tubes and none on the other tubes. The organism probably inhabits the perivascular spaces of the deeper portions of the brain, and the cultures and intravenous injections show that it probably does not grow well in body fluids.

SUMMARY OF BACTERIOLOGICAL FINDINGS

1. A staphylococcus-like organism has been isolated in primary pure cultures from six consecutive cases dying of encephalitis lethargica.

2. This organism when injected subdurally produces a fatal lethargic state in rabbits and the organism is recovered from the brain in pure culture.

3. The filtered culture contains a poison which produces a fatal lethargic state in rabbits.

4. Evidence is offered in favor of this effect being due to a poison (toxin?) generated by the growth of the organism rather than by a filterable virus.

5. Controls are reported to show that the lethargic state in animals was not due to the bouillon used for injection.

6. Agglutination experiments with patients' serum both in recovered and in sick cases are not productive of definite results, but are strongly suggestive.

In the August, 1919, number of this JOURNAL, C. M. Stafford[1] describes an organism obtained by spinal puncture of a lethargic patient which answers the same general description as the one we have isolated. Stafford's organism was nonpathogenic for rabbits intraspinously.

AUTOPSY FINDINGS

The autopsies were performed from one and one-half to three hours after death.

A summary of all the gross and microscopic findings will describe the pathologic picture better than to take up the individual protocols in detail. The intensity and distribution of the lesions varied somewhat and will be noted.

No abnormal external appearances of note are found. The scalp and tissues over the calvarium are normal. On removing the skull-cap there are no bone lesions. The dura is not adherent, and is white and glistening. The superior longitudinal sinus is empty. Upon removal of the dura we find the brain substance much redder than usual, due to the marked overfilling of the fine capillaries of the cortex. The pia is quite cloudy and looks thick and opalescent due to a marked edema of the leptomeninges. The superficial veins are distended. There are no gross hemorrhages, except very fine pin-point spots here and there on the cortical surface surrounding a tiny vessel.

The fluid in the cranial cavity is not usually increased, and the fluid in the ventricles is not much greater in amount than normal. The brain substance

itself gives a definite impression of being moister than normal and has a dough-like character.

On section all the vessels of the brain substance are found to be engorged and bleed freely from the cut ends. The spinal cord presents the same picture of congestion and distended venules, and, when handled with forceps, is definitely dough-like and soft.

The other organs of the body presented no changes of importance. In one case there was a terminal lobular pneumonia in an early stage of development. This case also had areas of focal necrosis in the liver. These areas were invaded by wandering cells and resembled closely those found in typhoid fever. The other cases did not show these areas and they are possibly due to intensive treatment with arsphenamine that this man at first received under the impression that he was suffering from cerebrospinal syphilis. The gastrointestinal tract and urinary tracts are normal. The heart has been normal in each case, and the lungs have showed occasional healed tubercles but were otherwise normal except for the case of terminal lobular pneumonia. One case had a pregnancy of about three weeks in utero. The sinuses in each case were thoroughly explored with negative results in all.

THE BRAIN LESIONS

Material was removed from the frontal, paracentral, calcarine, and hippocampal gyri, from the basal ganglia, thalamus, pons and medulla and cerebellum and fixed in formol, Zenker's, Cajal fluid and alcohol and stained by various methods.

The findings can best be classified according to location in meninges, cortex, basal ganglia, pons, medulla, and cord.

The meningeal findings are very characteristic and consist of marked edema of the pia and a moderate infiltration with wandering cells of different types. Neither the edema nor the infiltration is uniform in the leptomeninges, but is present in an irregular patchy manner. As in other meningeal inflammations, the findings are especially intense between the convolutions and the edema, and exudate dips down into the sulci.

The wandering cells consist of lymphocytes and plasma cells with large mononuclears scattered here and there. The infiltrating cells are usually scattered loosely through the edematous pia and do not tend to collect in dense foci except occasionally at the side of a vessel. The disease seems to stimulate a definite though feeble increase of the connective tissue, since young fibroblasts are present in the edematous infiltrated areas in increased numbers. The disease process in the affected areas of pia when dipping down into a sulcus are apparently definitely in relation to the perivascularly infiltrated vessels of the underlying cortical white matter.

The striking characteristic of the meningeal lesion is its apparent mildness.

The cortex: Some writers[2] have laid stress on the degenerative changes noted in the cortical pyramidal cells. Although Calhoun[3] lays more stress on the changes in the white matter at the base, Wegeforth and Ayer[4] found no changes of importance in the cortex. So far as our examinations go, we are

impressed with exactly the opposite picture. Taking the gray matter of the cortex as a whole, only occasionally is a cell found definitely degenerated. We are making the study of the finer changes in cell structure by special methods, a matter of a separate report, but judged from the work so far done, we believe that the degenerative changes in the cortex pyramidal cells are slight and unimportant. The tigroid substance is well seen for the most part. Only very rarely is a definite "satellitosis" and "neurophagia" seen and the blood vessels of the cortex, except occasionally in the white matter beneath a sulcus, are free from the characteristic perivascular infiltration so characteristic of the white matter of the basal portions of the brain. In the face of the very definite, and even destructive lesions of the lower portions of the brain, it seems to us unreasonable to ascribe undue importance to slight changes in the cortical pyramidal cells when many fields must be searched to find a degenerating cell. The cells of the gray matter of the cortex do not seem to us to show greater changes than cases of uremia or diabetes dying in coma. The white matter of the cortex and of the centrum semiovale shows an occasional small vessel with perivascular infiltration.

The basal ganglia: The changes in the basal ganglia, the caudate and lenticulate nucleus and the thalamus, are marked and consist of intense perivascular infiltration and perivascular edema of the white matter. Here again the lesion is essentially interstitial. The vessels of the white matter are involved and the ganglionic areas lying contiguous to the involved areas are affected secondarily by edema and occasionally by the margin of a small hemorrhage. The changes are in most cases most intense in the thalamus.

Pons and medulla and cord: Throughout the extent of the pons and medulla, the characteristic lesion is the perivascular infiltration of the white matter accompanied by edema and an occasional miliary hemorrhage. As we pass down the cord, the changes become less intense and in the cervical cord only an occasional vessel of the posterior horn is found infiltrated.

The pathologic findings may be summed up as follows:

1. Low grade leptomeningitis with edema and moderate round-cell infiltration.

2. Perivascular infiltration of the vessels of the white matter, especially of the caudate and lenticulate nuclei, optic thalamus, pons, medulla, and posterior horns of the cord with resulting edema and miliary hemorrhages of surrounding parts.

CLINICAL SIGNIFICANCE OF THE LESIONS

It seems to us that an *a priori* opinion could be arrived at regarding the essential nature of the lesion from a consideration of the clinical symptoms. In the first place the fact that, in spite of a profound lethargic state lasting over weeks, the patient is often restored to normal speaks decidedly against any extensive parenchymatous brain lesion. Degenerated brain cells that could be diagnosed by "neurophagia" etc., probably do not recover even if the patient lives, and we can not explain the return to normal on the basis of a destructive, degenerative lesion of the gray matter. The few degenerations found are probably due to edema and to injury of the nerve fiber as it passed outward through one of the

Fig. 5.—Infiltrated vessels on the posterior horn of the cervical cord.

Fig. 6.—Low power of cortex showing engorgement and rupture of pial veins.

involved areas lower down. Much might be said for this point of view as to the relative unimportance of the degenerative lesions of the Betz cells by an analysis of the mental state of the patients, but this question is more properly discussed in a clinical paper.

The involvement of the cranial nerves, probably the most constant single clinical manifestation of the disease is evidently due to the close admixture of white and gray matter in the pons and medulla. This point is well illustrated by the photographs which show areas of severely involved white matter contiguous to cranial nerve nuclei. We have been interested in the fact that we never find involvement of the motor cells of the anterior horn, but frequently find the vessels supplying the posterior root in the cord markedly affected. This finding correlates well with the fact that although the reflexes are frequently lost at the height of the disease, paralysis is not present. This finding, both pathologically and clinically, sharply distinguishes this affection from Heine-Medin disease.

In short "encephalitis lethargica" is not a true encephalitis in the sense that we speak of general paresis or the cerebral form of poliomyelitis as examples of encephalitis, because ganglion cell and pyramidal cell destruction does not characterize lethargic cases. But we are dealing here with a typical example of low grade "meningomyelitis" the characteristic lesions being in the meninges and white matter of the basal ganglia, pons, and upper cord. It has come to our attention through Dr. Camp of the University of Michigan that Marie in 1890 described cases similar to "encephalitis lethargica" and called them "acute multiple sclerosis." From a pathologic, as well as clinical, point of view, this term has much to justify its use.

BIBLIOGRAPHY

[1]Stafford, C. M.: Jour. Lab. and Clin. Med., iv, No. 11.
[2]Bassoe and Hassin: Arch. Neurol. and Psychiat., ii, 24.
[3]Calhoun, Henrietta A.: Ibid., iii, p. 1.
[4]Wegeforth and Ayer: Jour. Am. Med. Assn., lxxiii,, 5.
[5]Loewe and Strauss: Ibid., lxxiii, 1056.

PROTEIN FEVER
THE EFFECT OF EGG WHITE INJECTION ON THE DOG*

By Seymour J. Cohen, Chicago, Ill.

SPEAKING of his theory of fever, Victor C. Vaughan[1] and his coworkers reported some time ago that "It occurred to us, that if the above given ideas be correct, we should be able to induce in animals a continued fever by repeated subcutaneous injections of a soluble protein. We have done this with bacterial proteins, but since vitalistic dogmas cling so closely, we intend to present herewith an illustration of the result that we have obtained by repeated subcutaneous injections of egg white dilutions in rabbits * * * *.

"We think that these investigations show that fever results when a foreign protein is introduced into the blood. This foreign protein is not necessarily of bacterial origin, though under natural conditions this is usually the case in continued fevers."

In other words, these investigators conclude that repeated subcutaneous injections of a protein into animals produce a fever which is similar to the temperature curve found in typhoid fever. Friedberger[2] in 1910 while working on the relation between anaphylaxis and injection, also came to the same conclusion, that repeated parenteral injections of a protein produce a continued fever. Both of these investigators were able at will to produce a continued, intermittent or remittent fever, by varying the frequency and size of the injections.

This idea of the production of fever by parenteral injection of protein is not a new one. Roques, Gamallia, Charrin and Ruffer, Buchner, Krehl and Matthews and a score of other investigators have shown that parenteral injections of a foreign protein, bacterial or nonbacterial, may produce fever.

So far as could be determined, most of these former investigators did their work on either rabbits or guinea pigs. This present investigation was undertaken to review some of the earlier work but chiefly to determine the effect of parenteral injections of protein on larger animals, namely, the dog.

I. The Effect of Egg White Injections on the Guinea Pig

The same method was used as was described by Vaughan. A series of guinea pigs was weighed. A normal temperature was recorded for 10 days, then 2 c.c. of diluted egg white (one part egg white to one part .5 per cent phenol) were injected subcutaneously every 2 hours, over a period of 10 hours for 6 days. During this period the guinea pigs received a total of 60 c.c. of diluted egg white, injected 5 times a day. By examining Tables I and II and Figs. 1 and 2, it will be noted that the normal temperature of guinea pig I during the 10 days, varied between 97.3° and 100.1° and guinea pig II, between 98° and 100.7°. After the injection of the protein, the temperature began to rise on the second day and reached its maximum of 104.5° and 104.3,° respectively, on the sixth day, when

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the injections were stopped; after which the temperature gradually fell to normal. The animals themselves, showed all the signs of infection, during the high temperature. They were inactive and quiet; and remained in the corner of the cage huddled together. They did not eat much and consequently lost weight.

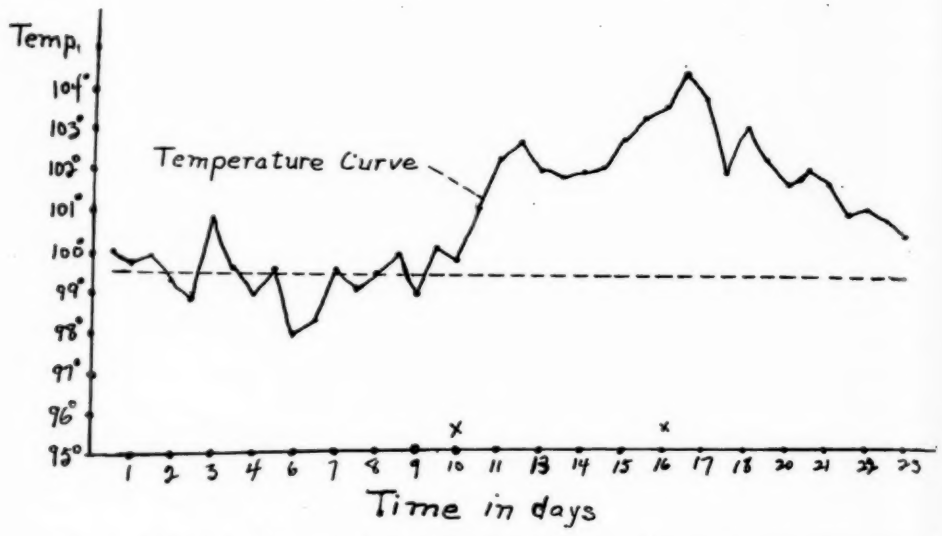


Fig. 1.—Showing the effect of subcutaneous injection of egg white on the temperature curve of Guinea Pig A.
X, injection begun; X, injections stopped.

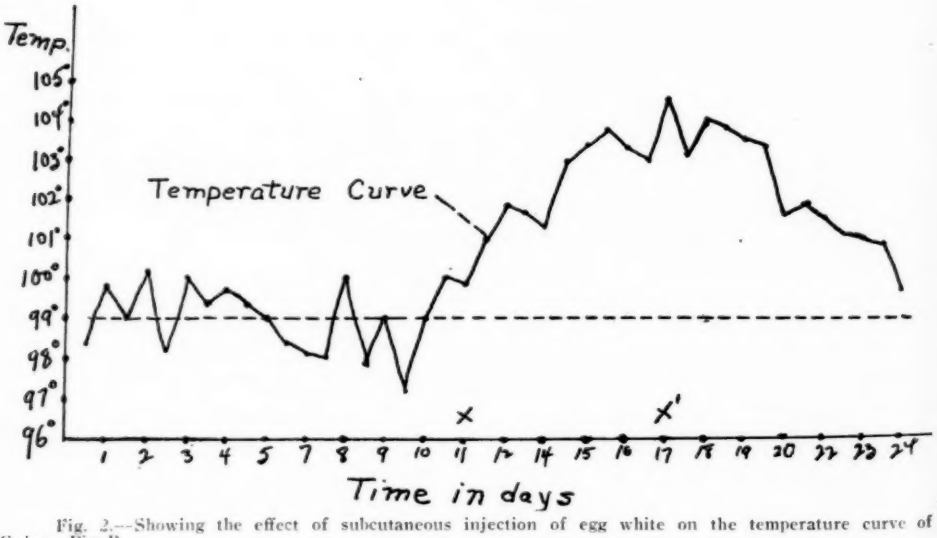


Fig. 2.—Showing the effect of subcutaneous injection of egg white on the temperature curve of Guinea Pig B.
X, injection begun; X', injections stopped.

These results agree quite uniformly with those of the previous investigators, that parenteral injection of protein in guinea pigs, at least, produces a continued fever.

II. THE EFFECT OF EGG WHITE INJECTIONS ON THE DOG

The injections of foreign protein into dogs were carried out along the same lines as those in the guinea pigs. A series of animals was prepared. Each animal was weighed and the urine examined before the experiment was begun. At

TABLE I

SUMMARY OF EXPERIMENT 1 SHOWING THE EFFECT OF SUBCUTANEOUS INJECTIONS OF EGG WHITE IN GUINEA PIG A

DATE	TEMPERATURE AND INJECTIONS					WEIGHT
	9:00	11:00	1:00	3:00	5:00	
5/14	100.0°				99.7°	370 gms.
5/15	99.8				99.4	
5/16	98.8				100.8	
5/17	99.6				99.8	
5/18						
5/19	99.6				98.	356 gms.
5/20	98.4				99.6	
5/21	99.2				99.5	
5/22	100.				99.0	
5/23	100.1				99.9	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/24	101.1				102.3	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/25						
5/26	102.7				102.	355 gms.
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/27	102.8				102.0	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/28	102.4				102.8	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/29	103.4				103.6	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/30	104.0				103.4	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/31	102.0				102.1	
6/1						
6/2	102.3				101.7	352 gms.
6/3	102.1				101.8	
6/4	102.0				101.	
6/5	101.3				100.	

TABLE II

SUMMARY OF EXPERIMENT 2 SHOWING THE EFFECT OF SUBCUTANEOUS INJECTIONS OF EGG WHITE IN GUINEA PIG B

DATE	TEMPERATURE AND INJECTIONS					WEIGHT
	9:00	11.00	1:00	3:00	5:00	
5/14	99.0°				100.1°	335 gms.
5/15	98.2				100.4	
5/16	99.4				99.6	
5/17	99.4				99.2	
5/18						
5/19	98.4				98.1	325 gms.
5/20	98.				100.2	
5/21	97.8				99.	
5/22	97.2				99.	
5/23	100.0				99.8	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/24	101.				101.8	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/25						
5/26	101.6				101.3	305 gms.
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/27	103.				103.4	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/28	103.7				103.3	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/29	103.1				104.5	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/30	103.1				104.0	
	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	
5/31	103.8				103.5	
6/1						
6/2	103.4				101.6	302 gms.
6/3	101.9				101.5	
6/4	101.1				101.	
6/5	100.9				99.8	

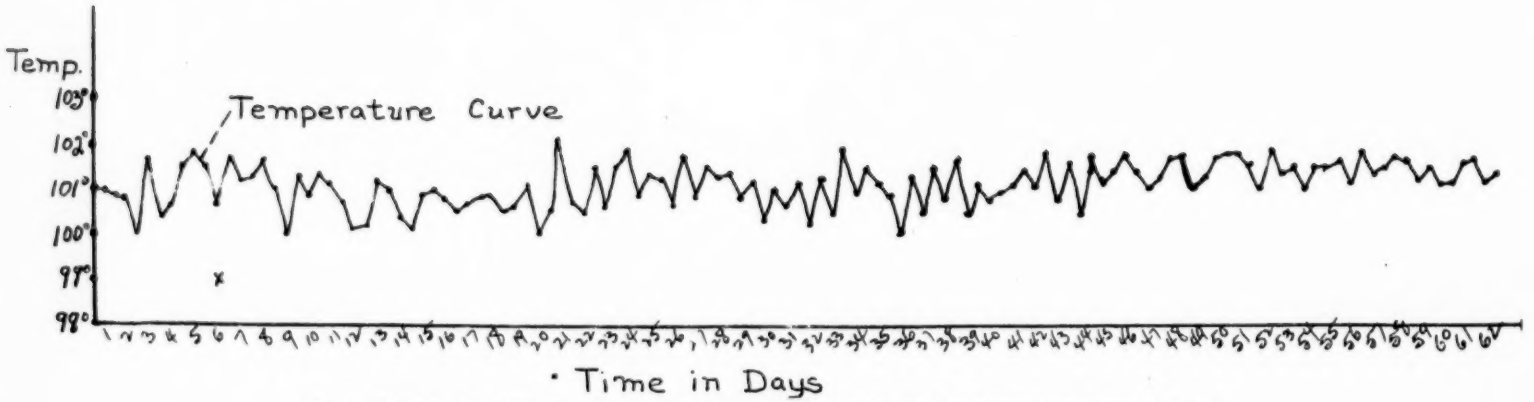


Fig. 3.—Showing the effect of subcutaneous injection of egg white on the temperature curve of Dog A. X, indicates beginning of injections.

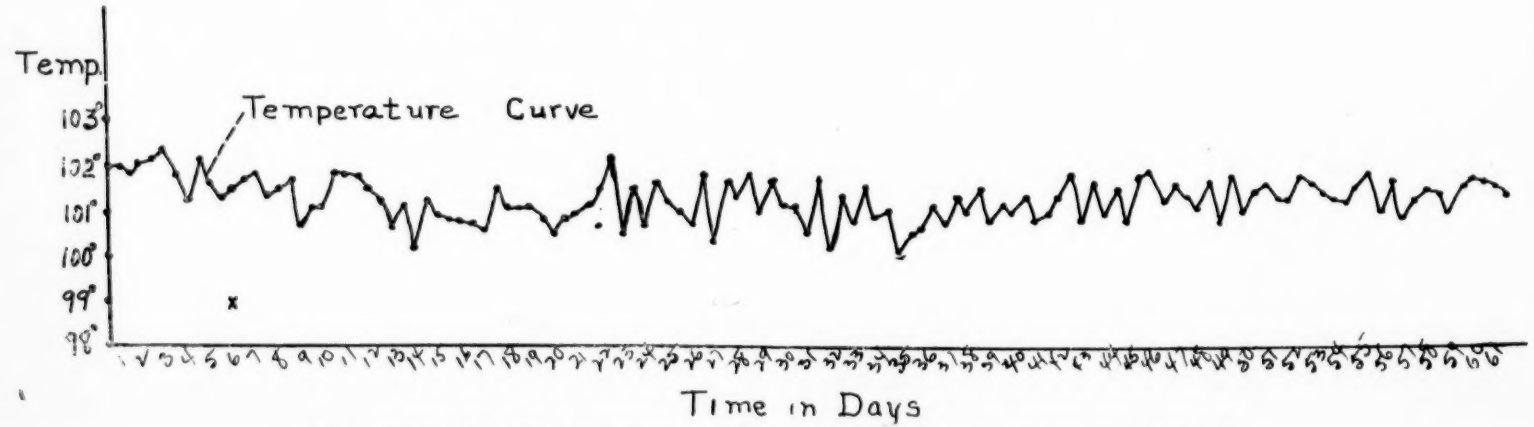


Fig. 4.—Showing the effect of subcutaneous injection of egg white on the temperature curve of Dog B. X, indicates beginning of injections.

the end of each week the dog was weighed and the urine reexamined, making in all 10 determinations.

Results.—As practically all the animals reacted in the same way, only results on two dogs will be described.

Dog I.—*Black and white spotted female dog, weight 7 kilos. Urine negative, contains no sugar or albumin.* A normal temperature was taken for 5 days, averaging about 101°. Then 3 c.c. of diluted egg white were injected subcutaneously 3 times a day for 3 days. This was followed by injection of 5 c.c. 4 times a day for 12 days. Then 8 c.c. were injected 5 times a day for 6 days. This was finally followed by injections of 10 c.c. of diluted protein 5 times a day for 36 days. This made a total of over 2300 c.c. of diluted egg white injected 4 and 5 times a day for a period of 57 days. By examining Chart 3 and Figure 3, it will be noticed that the temperature did not vary any more during the injections than before the injections. The temperature during the injection varied between 100° and 102.4°. As the normal temperature for this animal was around 101°, this variation can not be called fever. In addition it will be observed that the urine was always free from albumin and sugar except at one time, when there was a trace of albumin present. This animal gained in weight from 7 kilos to 8.1 kilos.

Dog. II.—*Brown female dog, weight* 5.1 *kilos. Urine negative.* A normal temperature was taken for 5 days averaging about 101.5°. The diluted egg white was injected subcutaneously in the same way as in the first animal. This animal also received over 2300 c.c. of diluted egg white, injected 4 and 5 times a day for a period of 57 days.

By examining Chart 4 and Figure 4, it will be seen that the temperature is practically uniform and analogous to the temperature curve of the first animal. In this case the temperature varied from 100.3° to 102.4°. This also can not be considered fever, when the average temperature of the animal before the injections was about 101.5°. The urine of this animal was at all times free from albumin or sugar. This animal gained in weight from 5.1 kilos to 6.3 kilos. The animals were not injected nor the temperature recorded on Sunday. At no time during the injections did the animals appear to be sick. They were always active and barking. The stools were well formed. There was never any diarrhea or any vomiting. During all these injections only one abscess developed, which lasted only a few days. Dog A is still alive and in good health at the present time, one year after the last injection. Dog B was also in good health several months after the last injection, when he was used for some crucial experiment.

These results on the series of dogs are practically identical and uniform with each other, but are so strikingly opposite from those obtained on the guinea pigs, that they deserved some consideration and discussion. Injections of egg white solutions (foreign proteins) produce no effect on the temperature curve of the dog. This is a fact. The egg white is absorbed from the place of injections, because the lump disappears in a few minutes. If the egg white injections were not absorbed, but became encapsulated and underwent fibrous changes; then the back of the dog should be nodular due to so many hundred injections, but this was not the case. The back of the animal, a few hours after the last injection, was as smooth and level as before the injections were begun.

The idea that the egg white in the dog is excreted without being broken down can be disproved by the fact that the urine in all cases was free from albumin. If the egg white was excreted by the bowel, it seems that there might be some intestinal disturbance, either a diarrhea or a severe constipation, but neither of these factors were present. The injected egg white was absorbed and must have been broken down in the tissue without producing any effect on the general constitution of the animal, chiefly a rise in temperature.

TABLE III

SUMMARY OF EXPERIMENT 3 SHOWING THE EFFECT OF SUBCUTANEOUS INJECTIONS OF EGG WHITE IN DOG A

DATE	INJECTIONS AND TEMPERATURE					WEIGHT	URINE
	9:00	11:00	1:00	3:00	5:00		
2/8	101.0°				100.8°	7 K	no albumin no sugar
2/9	100.8				100.0		
2/10							
2/11	101.6				100.4	7.3 K	no albumin no sugar
2/12	100.6				101.5		
2/13	101.8				101.5		
2/14	100.6 3 c.c.		100.8° 3 c.c.		101.7 3 c.c.		
2/15	101.2 3 c.c.		101.5 3 c.c.		101.3 3 c.c.		
2/16	101.6 3 c.c.		101.8 3 c.c.		101.0 3 c.c.		
2/17							
2/18	100.0 5 c.c.	100.1° 5 c.c.	100.4 5 c.c.	101.3° 5 c.c.		7.6 K	no albumin no sugar
2/19	100.9 5 c.c.	100.3 5 c.c.	100.5 5 c.c.	101.3 5 c.c.			
2/20	101.1 5 c.c.	101.3 5 c.c.	100.9 5 c.c.	100.6 5 c.c.			
2/21	100.1 5 c.c.	100.5 5 c.c.	101.0 5 c.c.	100.2 5 c.c.			
2/22	101.2 5 c.c.	100.9 5 c.c.	100.7 5 c.c.	101.0 5 c.c.			
2/23	100.4 5 c.c.	100.9 5 c.c.	100.5 5 c.c.	100.1 5 c.c.			
2/24							
2/25	100.9 5 c.c.	100.5 5 c.c.	100.3 5 c.c.	101.0 5 c.c.		7.5 K	no albumin no sugar
2/26	100.8 5 c.c.	101.0 5 c.c.	100.9 5 c.c.	100.5 5 c.c.			
2/27	100.7 5 c.c.	101.0 5 c.c.	100.9 5 c.c.	100.8 5 c.c.			
2/28	100.9 5 c.c.	101.0 5 c.c.	101.2 5 c.c.	100.5 5 c.c.			
3/1	100.7 5 c.c.	100.9 5 c.c.	101.0 5 c.c.	101.1 5 c.c.			
3/2	100.0 5 c.c.	100.6 5 c.c.	100.7 5 c.c.	100.5 5 c.c.			
3/3							
3/4	102.1 8 c.c.	101.9 8 c.c.	101.8 8 c.c.	101.6 8 c.c.	100.7 8 c.c.	7.8 K	no albumin no sugar
3/5	100.5 8 c.c.	100.7 8 c.c.	101.0 8 c.c.	101.6 8 c.c.	101.5 8 c.c.		
3/6	100.6 8 c.c.	101.8 8 c.c.	101.0 8 c.c.	101.6 8 c.c.	101.5 8 c.c.		
3/7	101.9 8 c.c.	101.7 8 c.c.	101.8 8 c.c.	101.6 8 c.c.	100.9 8 c.c.		
3/8	101.4 8 c.c.	100.9 8 c.c.	101.5 8 c.c.	100.8 8 c.c.	101.3 8 c.c.		
3/9	100.6 8 c.c.	101. 8 c.c.	100.9 8 c.c.	101.5 8 c.c.	101.8 8 c.c.		
3/10							
3/11	100.8 10 c.c.	101.2 10 c.c.	100.9 10 c.c.	101. 10 c.c.	101.5 10 c.c.	8 K	no albumin no sugar
3/12	101.3 10 c.c.	101.2 10 c.c.	101. 10 c.c.	100.9 10 c.c.	101.4 10 c.c.		
3/13	100.8 10 c.c.	101.2 10 c.c.	101.0 10 c.c.	101.1 10 c.c.	101.2 10 c.c.		
3/14	100.4 10 c.c.	100.9 10 c.c.	101.5 10 c.c.	100.6 10 c.c.	101. 10 c.c.		
3/15	100.6 10 c.c.	101.2 10 c.c.	101.1 10 c.c.	100.9 10 c.c.	101.2 10 c.c.		
3/16	100.3 10 c.c.	100.8 10 c.c.	100.5 10 c.c.	101. 10 c.c.	101.3 10 c.c.		
3/17							
3/18	100.5 10 c.c.	101. 10 c.c.	101.5 10 c.c.	101.7 10 c.c.	101.9 10 c.c.	8 K	trace of albumin no sugar
3/19	101. 10 c.c.	100.8 10 c.c.	101.0 10 c.c.	101.2 10 c.c.	101.5 10 c.c.		
3/20	101.2 10 c.c.	100.9 10 c.c.	101.0 10 c.c.	101.2 10 c.c.	100.9 10 c.c.		
3/21	100. 10 c.c.	100.3 10 c.c.	100.7 10 c.c.	101. 10 c.c.	101.3 10 c.c.		
3/22	100.5 10 c.c.	100.9 10 c.c.	101.0 10 c.c.	101.4 10 c.c	101.5 10 c.c.		
3/23	100.9 10 c.c.	101.3 10 c.c.	101.4 10 c.c.	101.8 10 c.c.	101.6 10 c.c.		
3/24							

TABLE III (CONT'D)

SUMMARY OF EXPERIMENT 3 SHOWING THE EFFECT OF SUBCUTANEOUS INJECTIONS OF EGG WHITE IN DOG A.

DATE	INJECTIONS AND TEMPERATURE					WEIGHT	URINE
	9:00	11:00	1:00	3:00	5:00		
3/25	100.5	100.6	101.	101.4	101.2		no albumin
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	7.8 K	no sugar
3/26	100.8	100.7	101.1	100.9	101.		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/27	101.1	101 5	101.3	101.2	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c	10 c.c.		
3/28	101.2	101.	101.2	101.7	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/29	100.8	101.4	101.6	101.9	101.6		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/30	100.5	100.8	101.4	101.1	101.8		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/31							no albumin
4/1	101.3	101.7	101.7	101.9	101.5		no sugar
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	8.1 K	
4/2	101.9	101.4	101.7	101.2	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/3	101.2	101.5	101.7	101.8	101.3		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/4	101.8	101.3	101.7	101.5	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/5	101.2	101.5	101.4	101.8	101.4		
	10 c.c.	10 c.c.	10 c.c	10 c.c.	10 c.c.		
4/6	101.8	101.5	101.0	101.6	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/7							no albumin
4/8	101.9	101.6	101.3	101.4	101.6		no sugar
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	8.2 K	
4/9	101.2	101.8	101.5	101.9	102.0		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/10	101.5	101.7	101.5	101.8	101.6		
4/11	101.2	101.1	101.4	101.4	101.6		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/12	101.4	101.0	101.4	101.2	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/13	101.3	101.5	101.9	101.6	102 0		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/14							no albumin
4/15	101.5	101.6	101.3	101.4	101.6		no sugar
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	8.3 K	
4/16	101.9	102.0	101.8	101.5	101.8		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/17	101.4	101.7	101.5	101.9	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/18	101.3	101.1	101.3	101.6	101.4		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/19	101.8	101.5	101.4	101.7	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/20	101.4	101.2	101.5	101.3	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		

Another factor which must not be overlooked is that of dosage. A guinea pig weighing approximately 250 grams received 2 c.c. of egg white, while a dog of about 7000 grams received only 10 c.c. In other words if the weight of the animal is taken as a basis, then the dog should have received twenty times as much, instead of only five times as much as the guinea pig. Consequently if this held good, then the dog should have received 40 c.c. of egg white five times a day. In the first place this amount is not only a huge one but also an unnecessary one. In the second place "Friedberger has in a most exact way confirmed the statement that large doses of foreign protein do not, while small doses do, elevate the temperature."

Why there should be a difference between the reaction in a guinea pig and the reaction in a dog to parenteral injection of proteins is difficult to explain. But this factor is apparent. A dog's temperature is much more constant than a guinea pig's or a rabbit's temperature. It is possible to increase the temperature of a guinea pig or rabbit by rough handling or by fright or excitement. In the

TABLE IV

SUMMARY OF EXPERIMENT 4 SHOWING THE EFFECT OF SUBCUTANEOUS INJECTIONS OF EGG WHITE IN DOG B

DATE	INJECTIONS AND TEMPERATURE					WEIGHT	URINE
	9:00	11:00	1:00	3:00	5:00		
2/8	102.				101.9°	5.1 K	no albumin
2/9	102.1°				102.2		no sugar
2/10							
2/11	102.4				101.8	5.2 K	no albumin no sugar
2/12	101.3				102.2		
2/13	101.7				101.4		
2/14	101.5		101.7°		101.6		
	3 c.c.		3 c.c.		3 c.c.		
2/15	101.8		101.6		101.4		
	3 c.c.		3 c.c.		3 c.c.		
2/16	101.5		101.7		101.6		
	3 c.c.		3 c.c.		3 c.c.		
2/17							
2/18	100.7	101. °	100.8	101.1°		5.4 K	no albumin no sugar
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/19	101.2	100.8	101.1	101.9			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/20	101.8	101.5	101.6	101.8			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/21	101.5	101.4	101.7	101.3			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/22	100.7	101.2	100.9	101.1			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/23	100.2	100.6	100.7	101.2			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/24							
2/25	101.0	100.8	100.5	100.9		5.7 K	no albumin no sugar
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/26	100.8	100.7	101.1	100.8			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/27	100.6	100.9	101.0	101.5			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
2/28	101.2	100.9	101.8	101.1			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
3/1	101.2	101.1	100.2	100.9			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
3/2	100.5	100.8	101.	100.9			
	5 c.c.	5 c.c.	5 c.c.	5 c.c.			
3/3							no sugar
3/4	101.	101.3	101.7	101.3	101.2	5.9 K	no albumin
	8 c.c.	8 c.c.	8 c.c.	8 c.c.	8 c.c.		
3/5	100.7	101.	101.3	100.9	102.2		
	8 c.c.	8 c.c.	8 c.c.	8 c.c.	8 c.c.		
3/6	100.5	101.2	100.9	101.6	101.5		
	8 c.c.	8 c.c.	8 c.c.	8 c.c.	8 c.c.		
3/7	100.7	101.0	100.9	101.3	101.6		
	8 c.c.	8 c.c.	8 c.c.	8 c.c.	8 c.c.		
3/8	101.3	101.	100.9	101.8	101.		
3/9	8 c.c.	8 c.c.	8 c.c.	8 c.c.	8 c.c.		
3/9	100.7	101.1	100.0	101.3	101.9		
	8 c.c.	8 c.c.	8 c.c.	8 c.c.	8 c.c.		
3/10							no albumin
3/11	100.4	100.7	101.	101.5	101.6	5.8 K	no sugar
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/12	101.4	101.4	101.2	101.	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/13	101.	101.0	101.3	101.4	101.6		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/14	101.2	101.9	101.8	101.3	101.1		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/15	100.5	101.	101.2	101.2	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/16	100.2	101.	100.9	101.1	101.4		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/17							no albumin
3/18	100.7	101.2	101.3	101.6	101.5	5.9 K	no sugar
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/19	100.9	100.6	100.9	100.7	101.0		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/20	100.1	100.5	100.7	101.0	100.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/21	100.6	101.	101.2	101.5	101.1		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/22	100.7	101.	101.0	101.1	101.3		
3/23	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/23	101.	101.7	101.2	101.1	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/24							
3/25	100.8	100.4	100.7	100.9	101.1	6.1 K	no albumin
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		

TABLE IV (CONT'D)

SUMMARY OF EXPERIMENT 4 SHOWING THE EFFECT OF SUBCUTANEOUS INJECTIONS OF EGG WHITE IN DOG B

DATE	INJECTIONS AND TEMPERATURE					WEIGHT	URINE
	9:00	11:00	1:00	3:00	5:00		
3/26	101.	100.9	101.3	101.1	101.3		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/27	100.8	101.0	100.8	101.1	101.0		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/28	101.4	101.5	101.3	101.6	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/29	100.8	101.0	101.2	101.4	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/30	101.	101.2	101.3	100.9	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
3/31							
4/1	100.8	101.0	101.3	101.6	101.8	5.9 K	no albumin
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		no sugar
4/2	102.	102.1	101.7	101.8	101.3		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/3	101.6	101.8	101.9	102.0	101.4		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/4	101.3	101.8	101.9	101.4	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/5	100.9	101.3	101.4	101.7	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/6	101.1	101.7	101.8	101.8	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/7							
4/8	101.7	101.4	101.6	101.5	101.4	6.2 K	no albumin
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		no sugar
4/9	101.4	101.9	101.3	101.7	101.9		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/10	101.7	102.	102.1	101.9	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/11	101.4	100.9	101.1	101.1	101.3		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/12	101.6	101.3	101.5	101.8	102.0		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/13	101.1	101.7	101.9	102.	101.8		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/14							
4/15	101.	101.4	101.3	101.6	101.4	6.2 K	no albumin
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		no sugar
4/16	101.6	101.8	101.7	101.3	101.5		
4/17	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/18	101.2	101.0	101.3	101.8	101.7		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/19	101.9	101.2	101.4	101.7	101.8		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		
4/20	101.7	101.5	101.6	101.8	101.5		
	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.		

dog the temperature center or heat center seems to be more constant and less affected by external stimuli. But even this does not explain the wide difference in the reactions of these animals. However, it remains a fact that repeated subcutaneous injections of egg white will not elevate the temperature of a dog.

SUMMARY

1. Repeated subcutaneous injections of egg white in guinea pigs produce a constant fever, associated with most of the signs of infection. (Confirmatory of Vaughan.)

2. Repeated subcutaneous injections of egg white in dogs do not affect the temperature curve and do not produce fever.

3. The author is unable to explain the difference of reactions.

ADDENDUM

The author of the above paper has asked the editor of the journal to make comment upon his article. The result obtained by Dr. Cohen is interesting. He

should now find out what becomes of egg white when injected subcutaneously in dogs. Is it changed in the blood or tissues, or is it eliminated through the liver into the intestines, there digested and absorbed? These matters can be decided by sensitizing guinea pigs with the blood and bile of dogs which have recently received subcutaneous injections of egg white.—Editor-in-chief.

BIBLIOGRAPHY

[1]Vaughan, Victor C.: Jour. Am. Med. Assn., 1909, liii, 629. Jour. Am. Med. Assn., 1911, lvii, 398. Jour. Lab. and Clin. Med., 1916, ii, 15. Protein Split Products, Phila., 1913, Lea and Febiger. Poisonous Protein, St. Louis, 1917, C. V. Mosby Co., p. 75.
[2]Friedberger: Berl. klin. Wchnschr, Oct. 1910, xlvii.
[3]Vaughan, Victor C.: Poisonous Protein, St. Louis, 1917, C. V. Mosby Co., p. 80.

LEUCOCYTES IN ANAPHYLAXIS OF SERUM SICKNESS

By Joseph H. Barach, M.D., Pittsburgh, Pa.

A RECENT contribution to the blood changes following intravenous injection of foreign protein, prompts me to record some observations made in a case of serum sickness with delayed anaphylactic reaction. The observations were made in 1912 and remained unpublished. But the findings of Cowie and Calhoun are so much in accord with mine that it seems worth while reporting them. Particularly so since Cowie and Calhoun consider the blood pictures which they found due directly to the intravenous injection, while my findings, similar to theirs, occurred in a case of subcutaneous injection. Also in their conclusions Cowie and Calhoun state the "temperature reaction and clinical findings are not in the nature of an anaphylactic response, as is shown by the absence of an eosinophilia" while the case reported here is a genuine anaphylactic response and eosinophilia was not present.

I point out these facts not in any way to contradict the conclusions, derived from their admirable work, but rather to offer one more ray of light on a complicated and interesting problem.

Patient, Miss E. K., taken ill October 29, 1912, with sore throat, muscle pains, and general symptoms of fever. Examination of throat revealed a diphtheritic infection and antitoxin was administered as the accompanying table and chart show:

TIME	AMOUNT OF SERUM	DIPH. UNITS	LOCAL REACTION
1st day of illness	1.5 c.c.	3,000	Slight
2nd day of illness	2.5 c.c.	5,000	Marked in 3 hrs.
4th day of illness	1.5 c.c.	3,000	Moderate

The tonsillar patches disappeared within 48 hours after first injection and recovery seemed to be progressing uneventfully.

On the morning of the 9th day after the first injection, patient complained of itching, and there was an urticaria over the site of the injection, which subsided after an alcohol rub. Fifteen hours later—on the tenth day of her illness—at 12:30 A.M., patient awakened out of her sleep with an itching over the entire body. The nurse proceeded to give her an alcohol rub, during which patient complained of being cold and chilly. Her skin was cold and clammy, her entire body became pallid, respirations shallow and accelerated, pulse small, rapid and for a short time could not be counted at the wrist. Temperature dropped to 97⅕°. External heat was applied, an injection of strychnine and one of pituitrin was given by the resident physician. In about half an hour patient reacted from this state of shock, and a diffuse urticaria appeared. First at the site of injection, then on the back, arms, lower extremities, face, palms of hands, soles of feet and abdomen. In some places the lesion consisted of small papules, in others, large blotches. The urticaria lasted for about twenty-four hours. Typical urticarial lesions limited to portions of the body, recurred during the two following days. They were evanescent, lasting a few minutes to an hour. Twelve hours after onset of the general urticaria, the pharynx and larynx became edematous; this was evidently due to the same process. The swelling in the throat continued for twenty-four hours.

At the time of this urticaria, patient also complained of pains in the elbow joints, and there was glandular swelling and pain in the neck and axilla.

By the twelfth day all symptoms had subsided and patient felt perfectly well.

It is important to note that the patient never had diphtheria or antitoxin prior to this, and that several years previous, a sister of hers had diphtheria, was given antitoxin and also experienced an attack of serum sickness.

We have here, then, a typical case of serum sickness with a delayed reaction, accompanied by anaphylactic shock and collapse which seemed to threaten the patient's life.

The blood findings are shown in the following table:

TABLE OF WHITE CELL COUNTS. (EHRLICH'S CLASSIFICATION)

TIME OF EXAM.	DAY OF DISEASE	LEUCOCYTES	POLY	LYMPHO.	LARGE MONO.	TRANS.	EOS.	MAST	MYELO-CYTES
17 hours after Anaphylactic Shock	10th	20,000 pr cum.	81.25	16.25	1.5	0.5	0.5	0.0	0.0
2 days later	12th	3,200 pr cum.	56.0	27.50	5.5	0.0	0.0	0.5	10.5
4 days later	16th	3,400 pr cum.	54.0	37.0	3.5	1.5	3.0	1.0	0.5
8 days later	20th	13,800 pr cum.	68.0	24.5	4.5	1.0	1.5	0.5	0.5

THE BLOOD FINDINGS

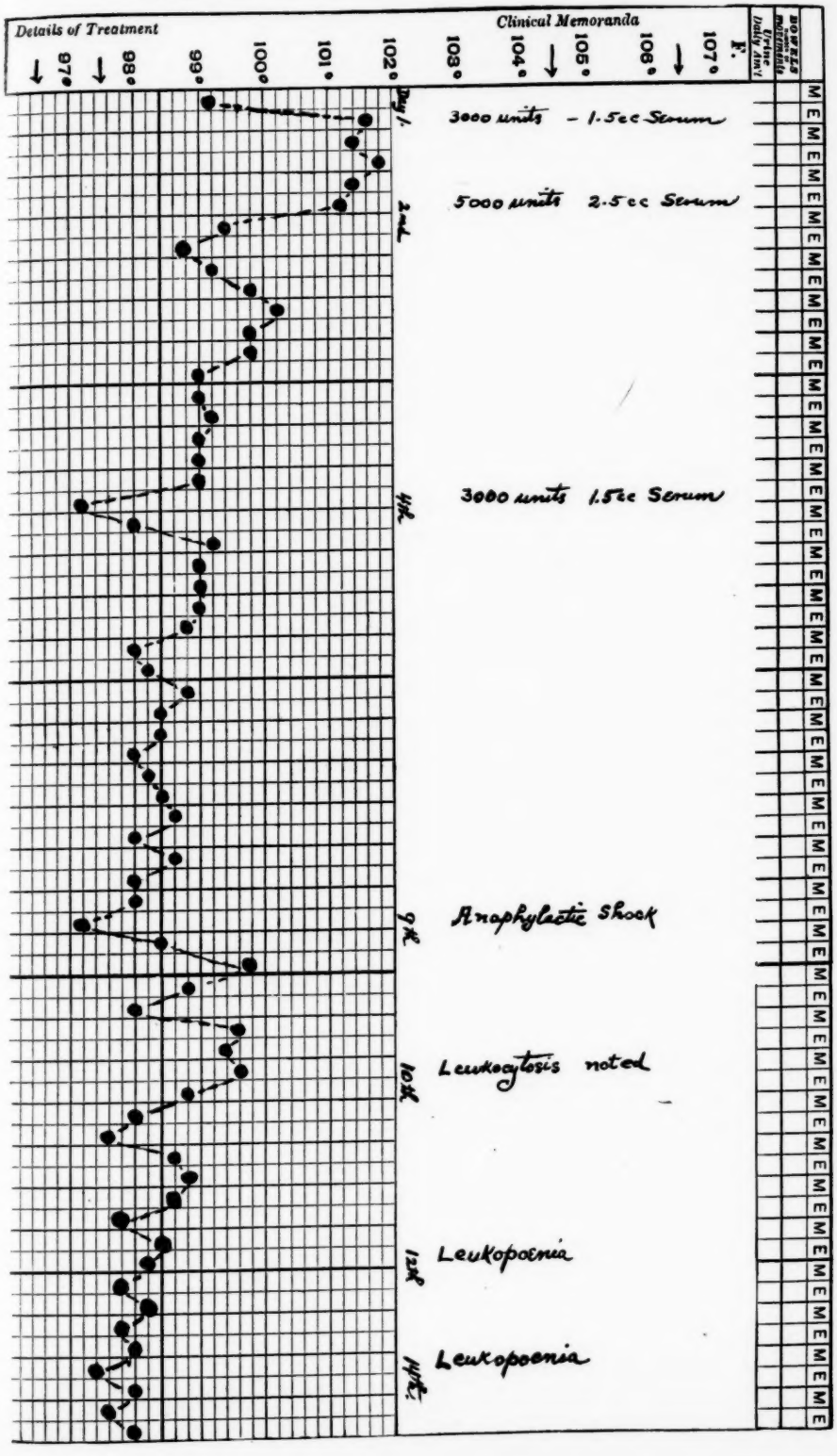
Considering that the temperature, pulse rate, and general condition of the patient seemed normal in every way preceding the anaphylactic attack, even though we have no record on this point, it seems fair to presume that the leucocyte count was about normal, or perhaps it is best to say that the blood picture was unknown.

Seventeen hours after the onset of the attack we find a polynuclear leucocytosis. This is followed by a leucopenia. Accompanying this leucopenia, or more likely at the end of the leucocytosis, we find the increased presence of large mononuclears, Ehrlich's myelocytes in rather large numbers, and the smear showed a marked increase in the blood platelets. Ehrlich's myelocytes are known to occur in the blood when there has been a call upon the reserve forces of the blood-making organs. The second count, two days later, still showed a leucopenia, but the myelocytes have disappeared, showing that their presence is not part of the leucopenia; more likely a precursor of it. The last count, eight days after the attack, was made when the patient had fully recovered. The count then showed a leucocytosis with the various cells in about normal proportions.

One phase of the admirable work of Vaughan[2] on cancer proteid—in which extensive observations including more than 20,000 differential counts were carried out—is confirmatory of the findings in this case.

By sensitizing rabbits to the cancer cell—and upon reinjection, producing anaphylaxis and in some instances shock—Vaughan found a transitory increase in the large mononuclears after each injection. This occurred 490 times in 500 animal experiments.

A noteworthy feature of the counts in this case, and in the anaphylactic reactions in Vaughan's work, is the absence of eosinophilia, which has been found present in a number of conditions said to be due to anaphylaxis.[3]


Details of Treatment
Clinical Memoranda
F.
97°
98°
99°
100°
101°
102°
103°
104°
105°
106°
107°
Day 1.
2nd.
4th.
9th.
10th.
12th.
14th.
3000 units - 1.5 cc Serum
5000 units 2.5 cc Serum
3000 units 1.5 cc Serum
Anaphylactic Shock
Leukocytosis noted
Leukopoenia
Leukopoenia

It is also worthy of note that in this case when the leucocytes had reached only 20,000 per cu. mm., the reserve forces of the blood supply had to be called upon; while in other diseases we may have a leucocytosis thrice this size without the appearance of myelocytes in the circulation. The probable reason seems to be that in anaphylaxis the demand is very sudden, whereas in other conditions, particularly in inflammations, the organism has more time to respond with matured white blood cells. The blood picture in this case of anaphylaxis at its critical period, in some respects resembles that of the crisis in cases of pneumonia, in which we have a preceding polynuclear leucocytosis, and at the time of the crisis, myelocytes and increased numbers of blood platelets.

SUMMARY

1. A case of serum sickness with a delayed anaphylactic reaction.

2. Blood shows at the time of the anaphylactic reaction, a primary polynuclear leucocytosis followed by the appearance of myelocytes after the organism had appropriated the available leucocytes of the circulating blood, and at the same time an increased number of blood platelets.

3. A leucopenia followed; at which time the polynuclear counts were low and the mononuclears relatively high.

4. The eosinophilia, which has been said to accompany anaphylactic reactions in general, was absent throughout.

5. Eosinophilia is not the criterion of an anaphylactic reaction.

BIBLIOGRAPHY

[1]Cowie and Calhoun: Non-Specific Therapy in Arthritis and Infections, Arch. Int. Med., Jan., 1919, p. 69.
[2]Vaughan, J. W.: Cancer Proteid, Jour. Am. Med. Assn., Nov. 16, 1912, p. 1764.
[3]Moschowitz: Eosinophilia and Anaphylaxis. New York Med. Jour., Jan. 7, 1911, p. 15.

STUDIES IN THE METABOLIC CHANGES IN EXPERIMENTAL TETANY*

By Dr. Tokuji Togawa, Tokyo, Japan

OUR present view as to the cause of tetany is contradictory. It is even undecided that the tetanic symptoms may be induced by a certain intoxication. Many facts in experimental tetany were studied and many theories in tetany were proposed by authors especially in America.

MacCallum and Voegtlin,[1] Medwedew,[2] Jacobson,[3] Underhill and Saiki,[4] Cooke,[5] Beebe and Berkeley,[6] Falta and Kahn,[7] Greenwald,[8] MacCallum and his coworkers,[9, 10] Wilson, Stearns, Janney and Thurlow,[11, 12] Burns and Sharpe,[20] Paton and Findlay,[13] Watanabe.[14,15]

The studies by Wilson, Stearns, Janney and Thurlow[11,12] showed that there is a condition of alkalosis after parathyroidectomy in dogs. This was proved by the studies on the dissociation of oxyhemoglobin, on the hydrogen-ion concentration of the blood, and on the carbon dioxide pressure of the alveolar air. They described further, that this alkalosis is neutralized by certain acids, probably by lactic acid formed during the tetanic period and in the next period an acidosis can be induced by overneutralization. Thus the periodic variations in the acid-base equilibrium cause the periodic attacks of tetany.

Studying on two dogs, parathyroidectomized, and on five dogs, subjected to gastric operation, W. S. McCann[16] found a marked increase in the carbon-dioxide-combining power of the blood plasma, coincident with the development of tetany.

In the recent studies by Watanabe,[15,16] it was demonstrated, that a marked acidosis appears in the condition of tetany caused by guanidine injection. He observed the same phenomenon in the parathyroid tetany and in idiopathic tetany.

The view seems therefore to be contradictory as regards the reaction of blood serum in experimental tetany.

So I have studied in the experimental puppies' tetany the carbon-dioxide-combining power of the blood plasma, the antitryptic power and the nonprotein nitrogen content of the serum.

The puppies were observed for three days at least and their normal condition assured. As method of operation the thyro-parathyroidectomy was chosen, because of its great simplicity and surety for removing all the parathyroid glands. Special attention was paid to leave a certain portion of the thyroid glands. All operative procedures were carried out without any narcotics.

1. Determination of the Carbon-Dioxide-Combining Power of the Blood Plasma

The apparatus of Van Slyke and Cullen[17] was used because of its great simplicity and accuracy. The blood sample was drawn usually from an ear vein directly into a paraffined centrifuge tube containing potassium oxalate. The

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plasma separated by centrifuging was saturated with alveolar air. The only variation from the original technic was the substitution of amyl alcohol for the caprylic alcohol. In the preliminary period the normal blood samples were obtained in three successive days. The average of the CO_2-combining power of these samples was taken as the normal value before operation.

2. DETERMINATION OF THE ANTITRYPTIC POWER OF THE SERUM

Bergmann and Meyer's method,[18] a little modified, was used, Gruebler's Trypsin sicc. being employed.

A. Titration of 0.1 per cent trypsin solution preceded the test proper. Into each of several test tubes were placed decreasing amounts of the trypsin solution as stated in Table I, A and II, A. To each tube 2.0 c.c. of 0.2 per cent casein solution were then added. After incubating for half an hour at 37° C., 1.0 c.c. of the acetic acid solution was added to each tube and the appearance of cloudiness was observed.

B. Determination of the antitryptic power of the serum. The fresh serum was diluted 20 times with salt solution. The mixtures of trypsin, casein, physiologic salt solution and serum were made as stated in Table I, B, I, C, and Table II, B. After incubating at 37° C. for half an hour 1.0 c.c. of acetic acid solution was added to each tube.

3. DETERMINATION OF THE NONPROTEIN NITROGEN CONTENT OF THE SERUM

Folin and Denis' method[19] was used. Five c.c. of the fresh serum were mixed with 50 c.c. of methyl alcohol. Twenty c.c. of the last filtrate were employed for nitrogen determination by Kjeldahl's method (duplicate determination).

PROTOCOLS

C = Vol. percentage of combined CO_2, calculated to 0°C., 760 mm.
anti-T = Antitryptic power of 1 c.c. serum.
N = Amount of nonprotein nitrogen in 100 c.c. of serum.

Dog 1, Bodyweight 1120 gm.
(i) Before operation.
XI, 16. C = 45.62%; anti-T = 20 (Table I, A and I, B)
N = 68.3 mg.
XI, 18. 10:30 A.M. operation.
(ii) After operation.
XI, 19. 4 P.M. slight stiffness of legs, walked not easy, no tremors.
XI, 20. Marked stiffness, mild tremor, depression, no walking.
11 A.M. blood drawn from carotid artery.
Animal sacrificed.
C = 37.51% (-8.11%); anti-T = 40 (Table I, A and I, C);
N = 94.5 mg. (+26.2 mg.)

Dog 2, Bodyweight 520 gm.
(i) Before operation.
XI. 19. C = 45.35%; anti-T = 40; N = 65.7 mg.
XI, 22. 1 P.M. operation.
(ii) After operation.
XI, 24. Afternoon, stiffness of legs.

XI, 25. 10 A.M. marked stiffness, especially of forelegs, hyperpnea, marked cramps time to time.
Immediately blood was drawn from carotid artery.
Animal dead.
C = 35.05% (−10.3%); anti-T = 60 (Table II, A and II, B);
N = 96.3 mg. (+30.6 mg.)

Dog 3, Bodyweight 720 gm.
(i) Before operation.
XI, 26. C = 49.0%; anti-T = 20; N = 70.1 mg.
XI, 27. 3 P.M. operation.
(ii) After operation.
XI, 28. Morning, stiffness.
XI, 29. Morning, shivering and contraction of the forelegs, exaggerated by stimulation, salivation. At 10:30 A.M. blood was drawn from femoral artery.
C = 36.4% (−12.6%); anti-T = 60; N = 94.6 mg. (+24.5 mg.)

Dog 4, Bodyweight 720 gm.
(i) Before operation.
XI, 28. C = 54.04%; anti-T = 40; N = 65.7 mg.
XI, 29. 4 P.M. operation.
(ii) After operation.
XII, 1. Stiffness of legs.
XII, 2. Morning. Violent tetany, twitching and jerking, hyperpnea, profuse salivation, all legs stiffly extended, moribund. Blood drawn, animal sacrificed.
Autopsy: only congestion of viscera.
C = 39.89% (−12.15%); anti-T = 60; N = 109.0 mg. (+43.3 mg.)

Dog 5, Bodyweight 560 gm.
(i) Before operation.
XI, 30. C = 51.4%; anti-T = 20; N = 100.7 mg.
XII, 2. 3 P.M. operation.
(ii) After operation.
XII, 4. Morning. Stiffness, slight tremor. Exertion caused contractions. At 10 A.M. blood was drawn.
C = 37.09% (−14.31%); anti-T = 40 (+20); N = 84.1 mg.

Dog 6, Bodyweight 720 gm.
(i) Before operation.
XII, 3. C = 47.37%; anti-T = 20; N = 68.3 mg.
XII, 4. 3 P.M. operation.
(ii) After operation.
XII, 5. Appeared bright, no sign of tetanic symptoms.
C = 47.38%.
XII, 6. Stiffness and tremors of legs, hyperpnea, salivation. Lying on its side with legs extended. Blood was drawn. Dog sacrificed.
C = 26.49% (−20.88%); anti-T = 60; N = 101.6 mg. (+33.3 mg.)

Dog 7, Bodyweight 1700 gm.
(i) Before operation.
XII, 18. C = 45.72%; anti-T = 40; N = 112.1 mg.
2 P.M. operation.
(ii) After operation.
XII, 20. Walking somewhat uneasy.
XII, 21. Morning. Violent tetany. Animal killed.
C = 37.48% (−8.24%); anti-T = 60; N = 91.1 mg.

TABLE I

THE ANTITRYPTIC POWER OF THE BLOOD SERUM BEFORE AND AFTER PARATHYROIDECTOMY (DOG 1)

A. TRYPSIN TITRATION					
	1	2	3	4	5
Trypsin	1.0	0.8	0.6	0.4	0.2
NaCl	0	0.2	0.4	0.6	0.8
Casein	2.0	2.0	2.0	2.0	2.0
Acetic Acid	1.0	1.0	1.0	1.0	1.0
	+	+	+	−	−

B. ANTITRYPTIC POWER BEFORE OPERATION						
	1	2	3	4	5	
Trypsin	1.0	0.8	0.6	0.4	0.2	
NaCl	0	0.2	0.4	0.6	0.8	
Casein	2.0	2.0	2.0	2.0	2.0	
Serum	0.2	0.2	0.2	0.2	0.2	
Acetic Acid	1.0	1.0	1.0	1.0	1.0	
	+	+	−	−	−	anti-T = 20

C. ANTITRYPTIC POWER AFTER OPERATION						
	1	2	3	4	5	
Trypsin	1.0	0.8	0.6	0.4	0.2	
NaCl	0	0.2	0.4	0.6	0.8	
Casein	2.0	2.0	2.0	2.0	2.0	
Serum	0.2	0.2	0.2	0.2	0.2	
Acetic Acid	1.0	1.0	1.0	1.0	1.0	
	+	−	−	−	−	anti-T = 40

(+) = Clearness
(−) = Cloudiness

TABLE II

THE ANTITRYPTIC POWER OF THE BLOOD SERUM AFTER PARATHYROIDECTOMY (DOG 2)

A. TRYPSIN TITRATION					
	1	2	3	4	5
Trypsin	1.0	0.8	0.6	0.4	0.2
NaCl	0	0.2	0.4	0.6	0.8
Casein	2.0	2.0	2.0	2.0	2.0
Acetic Acid	1.0	1.0	1.0	1.0	1.0
	+	+	+	−	−

B. ANTITRYPTIC POWER AFTER PARATHYROIDECTOMY						
	1	2	3	4	5	
Trypsin	1.0	0.8	0.6	0.4	0.2	
NaCl	0	0.2	0.4	0.6	0.8	
Casein	2.0	2.0	2.0	2.0	2.0	
Serum	0.2	0.2	0.2	0.2	0.2	
Acetic Acid	1.0	1.0	1.0	1.0	1.0	
	−	−	−	−	−	anti-T = 60

Dog 8, Bodyweight 610 gm.
(i) Before operation.
XII, 19. C = 46.77%; anti-T = 40. N = 112.1 mg.
2 P.M. operation.
(ii) After operation.
XII, 23. Morning. Tremor of all legs, would not eat, restless panting. At 11 A.M. Blood was drawn. Animal killed.
C = 34.81% (−11.96%); anti-T = 60; N = 161.2 mg. (−49.1 mg.)

Dog 9, Bodyweight 1100 gm.
(i) Before operation.
XII, 20. C = 44.77%; anti-T = 40; N = 66.6 mg.
XII, 21. 2 P.M. operation.
(ii) After operation.
XII, 23. Typical tetanic symptoms. Animal killed.
C = 51.99% (+7.22%); anti-T = 60; N = 84.6 mg. (+18.0 mg.)

Dog 10, Bodyweight 1220 gm.
(i) Before operation.
XII, 24. C = 44.45%; anti-T = 60; N = 69.0 mg.
2 P.M. operation.
(ii) After operation.
XII, 26. Walked about easily, appeared bright, slight stiffness of forelegs in excitement. Blood was drawn from ear vein.
C = 38.58% (−5.87%).
XII, 27. Morning. Violent cramps, restless groaning, moribund.
C = 35.55% (−8.9%); anti-T = 60; N = 94.6 mg. (+25.6 mg.)

Dog 11, Bodyweight 1460 gm.
(i) Before operation.
1,18. (1919) C = 45.35%; anti-T = 60; N = 49.0 mg.
4 P.M. operation.
1,20. (ii) After operation.
Slight stiffness of legs. C = 36.61% (−8.74%).
1,22. Stiffness scarcely noticed, walked easily.
C = 29.09% (−16.26%).
1,23. Bodyweight 1120 gm., marked stiffness N = 110.9 mg.
1,24. Stiffness and tremors, especially in excitement.
C = 28.26% (−17.09%).
1,25. C = 30.45% (−14.90%).
1,27. Bodyweight 1000 gm., weakness increased, legs quite relaxed, no appetite since yesterday, moribund. Blood was drawn from femoral artery. Animal dead.
C = 27.53% (−17.82%); anti-T = 60; N = 161.1 mg. (+112.1 mg.)

CONTROL TEST

Four dogs were thyroidectomized, special attention was paid to leave the parathyroid glands as intact as possible. In no cases tetanic symptoms were observed.

Dog 12, Bodyweight 1200 gm.
(i) Before thyroidectomy.
1,10. C = 37.04%; anti-T = 40; N = 56.0 mg.
1 P.M. thyroidectomized.
(ii) After thyroidectomy.
1,13. No sign of tetanic symptoms.
C = 36.96%; N = 54.0 mg. (− 2 mg.)
1,14. C = 44.64% (+7.60%); anti-T = 40; N = 50.2 mg. (−5.8 mg.)

1,15. almost normal. C = 37.60% (+0.56%).
1,16. Bodyweight 1040 gm; C = 44.98% (+7.94%); anti-T = 40; N = 56.0 mg.
1,17. Weakness and emaciation gradually increased, walked easily.
C = 44.76% (+7.72%).
1,18. C = 44.63% (+7.59%); anti-T = 60; N = 63.0 mg. (+7.0 mg.)
1,20. Since yesterday no appetite, Dog moribund. Blood was drawn from femoral artery. Animal killed.
Autopsy: Bronchopneumonia.
C = 38.49% (+1.45%); anti-T = 60; N = 105.1 mg. (+49.1 mg.)

Dog 13, Bodyweight 2320 gm.
(i) Before thyroidectomy.
1,13. C = 42.19%; anti-T = 40; N = 84.1 mg.
4:30 P.M. thyroidectomized.
(ii) After thyroidectomy.
1,17 Almost normal.
C = 46.0% (+3.81%); anti-T = 40; N = 49.0 mg. (-35.1 mg.)
1,18. No tetanic symptoms. C = 45.35%.
1,21. C = 48.32% (+6.13%); N = 49.0 mg. (-35.1 mg.).
1,22. C = 43.09% (+0.9%).
1,23. Bodyweight 2020 gm.; C = 48.98% (+6.79%); N = 52.9 mg. (-31.2 mg.)
1,24. C = 46.64%.
1,25. C = 47.23%.
1,27. Bodyweight 2000 gm.; C = 46.15%.
1,28. Bodyweight 1920 gm.; C = 41.64%. anti-T = 60.
1,31. Morning. Dog found dead. Autopsy: Bronchopneumonia.

Dog 14, Bodyweight 1420 gm.
(i) Before thyroidectomy.
1,21. C = 36.08%; anti-T = 60; N = 56.0 mg.
3 P.M. thyroidectomized.
(i) After thyroidectomy.
1,22. C = 44.22% (+8.14%).
1,23. Bodyweight 1320 gm.; C = 40.40% (+4.32%).
1,24. C = 33.95% (-2.13%); anti-T = 60.
1,25. C = 37.70% (+1.62%).
1,27. C = 39.14% (+3.06%).
1,28. Morning. Found dead.

Dog 15, Bodyweight 1440 gm.
(i) Before thyroidectomy.
1,22. C = 38.9%; anti-T = 60; N = 56.0 mg.
3:30 P.M. thyroidectomized.
(ii) After thyroidectomy.
1,23. C = 43.54%.
1,24. C = 39.72%; anti-T = 60; N = 59.5 mg.
1,25. C = 36.44%.
1,27. C = 38.17%.
1,28. C = 36.0%.
1,31. C = 38.64%. N = 91.1 mg.

From the preceding protocols or in the Table III it will be seen that the parathyroidectomized dogs, showing the tetanic symptoms, had not any sign of alkalosis. An acidosis, on the contrary, was observed almost constantly. The only exception was Dog 9, in which a slight alkalosis appeared after the parathyroidectomy.

TABLE III

THE CO_2 COMBINING POWER OF THE BLOOD PLASMA, THE ANTITRYPTIC POWER AND THE NONPROTEIN NITROGEN CONTENT OF THE BLOOD SERUM IN PARATHYROIDECTOMIZED DOGS

	C		ANTI-T		N	
DOG	BEFORE OP.	AFTER OP.	BEFORE OP.	AFTER OP.	BEFORE OP.	AFTER OP.
1	45.62	37.51	20	40	68.3	94.5
2	45.35	35.05	40	(60)	65.7	96.3
3	49.0	36.4	20	(60)	70.1	94.6
4	54.04	39.89	40	(60)	65.7	109.0
5	51.40	37.09	20	40	100.7	84.1
6	47.37	26.49	20	(60)	68.3	101.6
7	45.72	37.48	40	(60)	112.1	91.1
8	46.77	34.81	40	(60)	112.1	161.2
9	44.77	51.99	40	(60)	66.6	84.6
10	44.45	38.58 35.55	(60)	(60)	69.0	94.6
11	45.35	32.39	(60)	(60)	49.0	110.9 161.1

TABLE IV

THE CO_2 COMBINING POWER OF THE BLOOD PLASMA, THE ANTITRYPTIC POWER AND THE NONPROTEIN NITROGEN CONTENT OF THE BLOOD SERUM IN THYROIDECTOMIZED DOGS

	C		ANTI-T		N	
DOG	BEFORE OP.	AFTER OP.	BEFORE OP.	AFTER OP.	BEFORE OP.	AFTER OP.
12	37.04	38.92	40	40	56.0	55.8 105.1
13	42.19	45.93	40	40 (60)	84.1	50.3
14	36.08	40.48	(60)	(60)	56.0	
15	38.90	38.75	(60)	(60)	56.0	59.5 91.1

In the tetanic animals the antitryptic power of the serum was always increased. In Dogs 10 and 11, the antitryptic power of the serum was unusually strong, both before and after the operation.

As the time was not observed, any change of the antitryptic power due to the tetany can not be stated.

The amount of nonprotein nitrogen of the serum was usually increased by parathyroidectomy. Dogs 5 and 7, which had a rather high value of the nonprotein nitrogen content of the serum before the operation, did not show any increase.

In control animals, thyroidectomized but with a certain number of the intact parathyroid glands, acidosis could not be observed, but on the contrary a slight alkalosis condition was usually induced (Dogs 12, 13, 14). The antitryptic power and the non-protein nitrogen content of the serum did not show any marked variation. An increase observed directly before the death can not be considered to be related to the condition of athyreosis (Table IV).

SUMMARY

1. In parathyroidectomized dogs, showing typical tetanic symptoms, an acidosis condition is always observed.

2. The antitryptic power and the nonprotein nitrogen content of the blood serum are usually increased.

3. In thyroidectomized dogs, showing no tetanic symptoms, an acidosis condition is never observed. A slight alkalosis condition, on the contrary, is sometimes induced.

4. The antitryptic power and the nonprotein nitrogen content of the blood serum remain almost unchanged.

I desire to express my hearty thanks to Prof. S. Mita, to whom I am greatly indebted for his suggestions and help.

BIBLIOGRAPHY

[1]MacCallum, W. G., and Voegtlin, C.: Jour. Exper. Med., 1909, xi, 118.
[2]Medwedew, A.: Ztschr. f. physiol. Chem., 1911, lxxii, 410.
[3]Jacobson, C.: Am. Jour. Physiol., 1910, xxvi, 407.
[4]Underhill, F. P., and Saiki, T.: Jour. Biol. Chem., 1908-09, v, 225.
[5]Cooke, J. V.: Jour. Exper. Med., 1911, xiii, 439.
[6]Berkeley, W. N., and Beebe, S. P.: Jour. Med. Research, 1909, xx, 149.
[7]Falta, W., and Kahn, F.: Ztschr. f. klin. Med., 1911, lxxiv, 108.
[8]Greenwald, I.: Jour. Biol. Chem., 1913, xiv, 363 and 369.
[9]MacCallum, W. G., and Vogel, K. M.: Jour. Exper. Med., 1913, xviii, 618.
[10]MacCullum, W. G., Lambert, R. A., and Vogel, K. M.: Jour. Exper. Med., 1914, xx, 149.
[11]Wilson, D. W., Stearns, T., and Janney, J. H.: Jour. Biol. Chem., 1915, xxi, 169.
[12]Wilson, D. W., Stearns, T., and Thurlow, M. DeG.: Jour. Biol. Chem., 1915, xxiii, 89.
[13]Paton, D. N., and Findlay, L.: Quart Jour. Exper. Physiol, 1916, x, 203 and 315.
[14]Watanabe, C. K.: Jour. Biol. Chem., 1918, xxxiii, 253.
[15]Watanabe, C. K.: Journ. Biol. Chem., 1918, xxxiv, 65, 73 and 51.
[16]McCann, W. S.: Jour. Biol. Chem., 1918, xxxv, 553.
[17]Van Slyke, D. D., and Cullen G. E.: Jour. Biol. Chem., 1917, xxx, 317.
[18]Bergmann and Meyer, K.: Berl. klin. Wchnschr., 1908, xlv, 1673.
[19]Folin, O., and Denis, W.: Jour. Biol. Chem., 1912, xi, 527, ibid., 1913, xiv, 29.
[20]Burns, D., and Sharpe, J. S.: Quart. Jour. Exper. Physiol., 1916, x, 345.

BACILLUS BRONCHISEPTICUS AS THE CAUSE OF AN INFECTIOUS RESPIRATORY DISEASE OF THE WHITE RAT*

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THE importance of Bacillus bronchisepticus as a pathogenic microorganism has been firmly established by the investigations of numerous workers, both in this country and abroad. A search of the literature dealing with the bacteriology of various diseases of the smaller animals, for the past thirty years, leaves no doubt that B. bronchisepticus was frequently encountered by the investigators who studied these diseases. In the majority of the reports the completeness of the data does not permit a positive statement, now, to the effect that this or that organism was the same as the one we now call B. bronchisepticus, but the fragmentary evidence dealing with the morphology and cultural characteristics, together with certain statements as to the symptoms shown by the infected animals, really leaves little room for doubt as to the identity of some of these organisms with B. bronchisepticus, especially in the light of our present knowledge regarding it and its pathogenic powers.

BRONCHISEPTICUS INFECTIONS OF OTHER ANIMALS

It remained for Ferry[1] to publish the first complete description of the organism, to which he first gave the name B. bronchicanis, having isolated it from dogs suffering with distemper. In pursuing his investigations further, Ferry found that the organism did not confine its pathogenic activities to the dog, but that it was associated with distemper-like diseases of other small animals. This caused Ferry[2] to rename the organism B. bronchisepticus. This work was confirmed by the publications of M'Gowan[3] and Torrey and Rahe,[4] which followed shortly after Ferry published his findings. Up to this time B. bronchisepticus has been reported as having been isolated from dogs, cats, rabbits, guinea pigs, ferrets, monkeys and man, but not from rats. M'Gowan[3] made bacteriologic examinations of rats in his laboratory, while studying infections among his laboratory animals, but did not find B. bronchisepticus in cultures made from the lungs, trachea, back of the nose and heart blood.

It is the purpose of this report to record the finding of B. bronchisepticus in pure culture as the apparent cause of a serious distemper-like disease among white rats. The white rat (Mus Norwegicus albinus) is now being extensively used in certain laboratory investigations, especially in work with glandular substances, vitamines and other accessory food substances, in the study of dietary deficiency diseases. In many laboratories the rats are kept in the same buildings with other small animals, such as guinea pigs and rabbits, or even in close proximity to dog kennels, thereby affording favorable conditions for the spread of such an infection as B. bronchisepticus, probably the most common pathogenic organism found among laboratory animals.

*From the Research Laboratories, Parke, Davis & Co., Detroit, Michigan.

NATURE OF THE DISEASE

The symptoms shown by affected rats are rather constant. These include loss of appetite, dullness, loss in weight, and respiratory disturbances. The latter are quite characteristic and sometimes are present without the other symptoms mentioned. There is a nasal discharge, which varies in consistency, color and amount, in different cases. Accompanying this are frequent paroxysms of sneezing and a peculiar "rattling" breathing sound. For this symptom the name "snuffles" has been casually applied by laboratory attendants, probably on account of the similarity of the rat disease with the well-known "snuffles" of rabbits. Diarrhea was not noted in any of the cases observed.

The disease may run a protracted course or end suddenly. Few recoveries have been noted. Usually a pneumonia has been found in those cases terminating fatally. No attempt has been made to treat cases of the disease for obvious reasons. It has been on the part of economy to destroy all rats showing symptoms and start over again with new stock, placed in thoroughly cleaned and disinfected quarters. There have been times when it was very difficult to obtain healthy stock from commercial rat-breeding establishments. Shipments have been made of apparently healthy stock, only to have them arrive with some of the rats showing symptoms.

BACTERIOLOGIC EXAMINATIONS

Material for study consisted of sick rats in various stages of the disease and rats dead of the infection. Cultures were made by plating material from the upper respiratory tract, trachea, lungs, and heart blood. Table I shows that B. bronchisepticus was isolated from two rats dead of the infection, and from eleven others killed for autopsy and cultural purposes. The organism was isolated from the nostrils, nasal sinus, trachea, lungs, and heart blood. Usually the cultures from the trachea were pure, as well as those from the lungs and heart blood in the two cases where the organism was recovered from these sources. Cultures made from the upper respiratory tract, nostrils and nasal sinuses, frequently

TABLE I

DATA ON RATS FROM WHICH B. BRONCHISEPTICUS WAS ISOLATED

RAT NO.	KILLED OR DIED	SYMPTOMS	ORGANISM
2	K	Sick; "rattling" breathing	Sinus
3	K	Sick; "rattling" breathing	Sinus
4	K	Sick; "rattling" breathing	Sinus
7	K	Sneezing, "rattling" breathing	Trachea
14	K	Sneezing	Nostril
19	D	Not observed	Heart Blood*
21	K	Sneezing	Trachea*
24	K	Sneezing	Trachea*
25	K	Sneezing	Trachea*
26	K	Sneezing	Trachea*
28	K	Sneezing	Sinus
29	K	Sneezing	Sinus
31	D	Not observed	Lung*

*B. bronchisepticus in pure culture.

yielded B. bronchisepticus, as well as other organisms, such as staphylococci and pyocyaneus. Gas producers were not found.

At the same time that rats Nos. 20-29 were killed, samples of blood were taken, the serum removed and subsequently used for a series of agglutination tests, against B. bronchisepticus isolated from rats in the present outbreak, as well as cultures of the organism obtained from dogs. (See Table II.)

In the isolation and identification of B. bronchisepticus, suspected colonies

TABLE II

AGGLUTINATION TESTS

(1) B. bronchisepticus (rat) suspensions against homologous rat sera.
(2) B. bronchisepticus (rat)* suspensions against rat sera.
(3) B. bronchisepticus (dog) suspensions against rat sera.
*(Strain obtained from Rat 31)

(1) Suspension of B. bronchisepticus against the serum of the rat from which the strain was isolated.

DILUTIONS	RAT 21	RAT 25	RAT 28
1-8	++	+++	+++
1-16	+	++	+++
1-32	–	++	+++
1-64	–	++	++
1-128	–	++	+
1-256	–	+	+
1-512	–	+	–
1-1024	–	–	–
Controls	–	–	–

(2) Suspension of B. bronchisepticus (Rat 31) against sera of rats sick with disease.

DILUTIONS	RAT 21	RAT 22	RAT 23	RAT 24	RAT 25	RAT 26	RAT 27	RAT 28	RAT 29
1-8	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-16	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-32	++	++	+++	+++	+++	+++	+++	+++	+++
1-64	++	++	++	++	++	++	++	++	++
1-128	++	++	++	++	++	++	++	+	++
1-256	+	++	++	+	+	+	+	+	+
1-512	–	+	+	–	–	–	-	–	+
1-1024	–	–	–	–	–	–	–	–	–
Controls	–	–	–	–	–	–	–	–	–

(3) Suspension of B. bronchisepticus (Canine Strain) against sera of rats sick with disease.

DILUTIONS	RAT 20	RAT 21	RAT 22	RAT 23	RAT 24	RAT 25	RAT 26	RAT 27	RAT 28	RAT 29
1-8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-16	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1-32	+++	+++	++	+++	+++	++	+++	+++	+++	+++
1-64	++	++	++	+++	++	++	++	++	++	+++
1-128	++	++	++	++	++	+	++	++	++	++
1-256	++	+	+	++	+	+	+	++	+	++
1-512	++	+	+	–	+	+	–	++	–	+
1-1024	++	–	–	–	–	–	–	–	–	–
Controls	–	–	–	–	–	–	–	–	–	–

were picked from the agar plates and transferred to various media. All organisms which were ultimately designated B. bronchisepticus failed to produce gas or acid in fermentation tubes containing dextrose, lactose, and saccharose bouillon. The closed arm remained clear with turbidity in the open arm. Litmus milk was permanently alkalined in 48 to 72 hours, and the growth on potato was yellowish brown (tan). All organisms were actively and progressively motile, Gram-negative, nonspore forming, and failed to produce indol.

DISCUSSION

It will be noted that with two exceptions the agglutination titers were fairly uniform. One exception, where the serum of Rat 21 was run against the homologous strain of B. bronchisepticus, the suspension did not show any agglutination above a dilution of 1 to 16. The same serum agglutinated a strain of the organism from another rat at a dilution of 1 to 256. The organism from Rat 21 was typical of B. bronchisepticus in all respects. The other exception was in the case of the serum from Rat 20, which strongly agglutinated a canine strain of the organism at a dilution of 1 to 1024. At autopsy this rat showed pneumonic areas in the lungs. We were unable to determine the agglutination titer of the serum of normal rats. All rats on hand had been exposed to the disease and efforts to locate healthy stock were unsuccessful. Serum from normal rabbits does not usually agglutinate B. bronchisepticus above a dilution of 1 to 10.

SUMMARY

1. Bacillus bronchisepticus has been isolated from nostrils, nasal sinuses, trachea, lungs and heart blood of white rats affected with a serious disease of a distemper-like character.

2. The organism was recovered in pure culture in about one-half of the cases. Other organisms were found with B. bronchisepticus, in the nostrils and nasal sinuses, and once in the trachea.

3. Agglutination tests pointed to the identity of the rat organism and B. bronchisepticus from a canine source.

4. The serum of rats affected with the disease agglutinated both homologous as well as heterologous strains of B. bronchisepticus in comparatively high dilutions. One rat serum showed strong agglutination at a dilution of 1 to 1024.

BIBLIOGRAPHY

[1]Ferry, N. S.: A Preliminary Report of the Bacterial Findings in Canine Distemper, Am. Vet. Rev., 1910, xxxvii, No. 4, pp. 499-504.

[2]Ferry, N. S.: Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper, Am. Vet. Rev., 1912, xli, No. 1, pp. 77-79.

[3]M'Gowan, J. P.: Some Observations on a Laboratory Epidemic, Principally Among Dogs and Cats, in Which the Animals Affected Presented the Symptoms of the Disease Called "Distemper," Jour. Path. and Bact., 1911, xv, No. 5, pp. 372-426.

[4]Torrey, J. C., and Rahe, A. H.: Studies in Canine Distemper, Jour. Med. Res., 1913, xxvii, No. 3, pp. 291-364.

BACTERIOLOGY AND CONTROL OF CONTAGIOUS NASAL CATARRH (SNUFFLES) OF RABBITS*

By N. S. Ferry, Ph.B., M.D., and H. Preston Hoskins, V.M.D., Detroit, Michigan

THE condition among rabbits commonly referred to as "snuffles" has been, more or less, a disturbing element to laboratory workers ever since the animal in question was first used so generally for experimental purposes. It has also of late bid fair to become of considerable economic importance, as the practice of breeding and raising rabbits by many individuals in this country as a means of augmenting the family exchequer has recently developed into an industry of extensive proportions.

On account of the general distribution of this industry and the prevailing custom of exhibiting pure bred rabbits at the meetings of the various state and local rabbit associations, together with the opportunity offered for the spread of the infection in pet shops and bird stores, the disease has necessarily become rapidly and widely disseminated. This has resulted in an increasing demand for a more comprehensive bacteriologic study of the infectious process, as it now exists, and the development, if possible, of a means of protection against it.

PREVIOUS BACTERIOLOGIC FINDINGS

One of the earliest descriptions of an epizootic, of bacterial origin, of the respiratory tract of rabbits was given by Beck[1] in 1893. He described an infectious rabbit disease accompanied by a catarrhal pneumonia and often a fibrinous pleuritis caused by a small bacillus which was aerobic, nonmotile, negative to Gram and which could not be grown on potato, although it thrived on other alkaline media. This bacillus was found to kill rabbits within five days after intrapulmonary inoculations. It took eight or ten days, however, to kill after inoculations directly into the blood or on the uninjured nasal mucous membranes. The organism proved pathogenic for mice and guinea pigs.

In 1897 Kraus[2] described an epizootic in rabbits which resulted in pneumonia with purulent pleuritis and pericarditis, accompanied by a purulent nasal catarrh and an inflammation of the antrum of Highmore. The infection was produced by a small, Gram-negative, aerobic, motile bacillus which developed a yellowish-brown growth on potato.

Volk[3] in 1902 described a contagious disease of rabbits which always appeared to attack the pleura, pericardium and endocardium. As the cause of the infection Volk reported a small, aerobic, nonmotile bacillus, negative to Gram, which was able to grow luxuriantly on potato. This organism appeared to be extremely virulent, killing rabbits within 36-48 hours with a dose of 0.000001 c.c. of a 24-hour bouillon culture after an intraperitoneal or intrapleural inocula-

*From the Research Department, Parke, Davis & Co., Detroit, Mich.
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tion. This amount inoculated into the uninjured nasal membranes took three days to kill, while 0.005 c.c. injected into the trachea resulted in death within ten days.

Südmersen in 1905[4] described an infectious rabbit pneumonia in which a fibrinous pleuritis and catarrhal lung inflammation appeared. The cause of the disease was a short, thin, occasionally double, actively motile bacillus which was Gram-negative and easily stainable with the ordinary aqueous aniline dyes. On potato it formed a yellowish-brown, wax-like growth and produced gas and indol.

In 1910 and 1911 one of us (N. S. F.)[5, 6] described an organism as causing distemper in dogs and later, 1912[7, 8] and 1914,[9] as producing snuffles in rabbits and a disease similar to distemper in other small animals. This organism, which the above author ultimately named Bacillus bronchisepticus, is recognized by the following characteristics: It is a short, slender bacillus, usually found single, but often in pairs. In liquid media it may be seen in long chains or filaments and, when cultivated directly from the animal body, may be larger and more oval in form. It does not stain by Gram but stains with most aniline dyes and with Löffler's methylene blue often gives a typical bipolar appearance. The organism is actively and progressively motile. It gives a filiform translucent growth on solid media. On potato and Koch's serum it produces a tan colored moist growth. It does not liquefy gelatin, and does not produce acid or gas in sugar. It gives an alkaline reaction to litmus milk. In broth it produces a persistent clouding with a rather viscid sediment. Indol is negative.

The disease as it was found in rabbits was briefly described by Ferry[9] as follows: "Loss of appetite and flesh with decreased activity are usually the initial symptoms. Diarrhea is an invariable symptom, while a discharge from the nose and eyes (purulent very early) is recognized in most cases; wherein it differs from the guinea pig. Death in the majority of cases is found in from two to ten days, although the disease is not as fatal as with the guinea pig. Incubation period from five to seven days. In the early stages B. bronchisepticus is usually found in the respiratory tract in pure culture. It may also be found in the blood and abdominal organs. In later stages of the disease this microorganism is associated with pyogenic organisms of secondary infections."

Ferry also mentioned an uncommonly acute infection which was present among his rabbits, at the same time, due to a bacillus of the hemorrhagic septicemia type. This disease was of extremely short duration and invariably fatal. "Often a slight discharge was observed at the nostrils but this was not so profuse nor so purulent as in snuffles due to B. bronchisepticus. At autopsy a general invasion of the body with the specific microorganism was the rule." An observation was made at that time which appears to have been substantiated by later facts that "slight discharge (of the nostrils) seems to be a common symptom of most infections in the rabbit."

In 1911 M'Gowan[10] described an organism found in an epidemic among laboratory animals, including the rabbit, (presenting the symptoms of the disease called distemper) which proved identical to the organism just preceding (B. bronchisepticus). The condition among M'Gowan's rabbits was, no doubt, typical snuffles.

In 1913 and 1917 Davis[11, 12] described a Gram-negative, nonmotile, polar staining minute bacillus which was found in subcutaneous abscesses and in snuffles in rabbits.

PRESENT INVESTIGATIONS

Culture material was obtained from rabbits of various ages and during all stages of the disease, particular attention being paid, wherever possible, to rabbits showing early symptoms of the disease. All cultures were obtained by streaking the material on plates or planting in broth. Previous work, as well as preliminary experiments during the present investigations, showed that the predominating and most consistent colonies were small, round, grayish, translucent and small, round, white, opaque. These two types alone were employed for isolation work. Smears from other colonies were studied, however, but the findings proved conclusively that they had nothing in common with the primary infectious process.

During the life of the rabbits, cultures were obtained from the nares by passing sterile swabs as far into the nasal cavity as possible. At autopsy cultures were obtained from the nasal sinuses, trachea, heart's blood and various organs of the body.

Access was afforded the investigators for a thorough study of this disease as it presented itself in rabbitries in various parts of the country and under conditions ideal for such a line of work, so that the conclusions are in no way influenced by conditions prevailing in any one locality. Entire rabbitries were placed at the disposal of the investigators for bacteriologic observations as well as for further experimental work, and as a result, a large number of animals were studied either experimentally or clinically. With these rabbits, also, were included many which were under observation for the effect of specific bacterial vaccine treatment, both prophylactic and therapeutic.

RABBITS STUDIED BACTERIOLOGICALLY

1. Private stock (6 mos. old doe, Flemish Giant). June 16, 1919. Symptoms 7 months. Thick discharge from nose; sneezing. Cultures from nares. Numerous colonies of a small bacillus (B. bronchisepticus) and a few colonies of Staph. albus.

2. Private stock (young buck, Flemish Giant). June 16, 1919. Sick four weeks. Thick discharge from nose; sneezing. Cultures from nares. Numerous colonies of a small bacillus; B. bronchisepticus in large numbers; few Bact. lepisepticum and few Staph. albus.

3. Private stock (young doe, Flemish Giant). June 16, 1919. Just showing signs of illness; slight watery discharge from nose. Cultures from nares. Small bacillus in large numbers; plate contaminated; B. coli only organism isolated.

4. Private stock (young doe, Flemish Giant). June 16, 1919. Sneezing 3 to 4 days. Cultures from nares; small bacillus in large numbers which proved to be B. bronchisepticus; Staph. albus also isolated.

5. Private stock (buck, Flemish Giant). June 19, 1919. Sick six months; thick discharge. Cultures from nares. Several organisms present; gas producing organisms of the colon type isolated.

6. Private stock (young doe, Flemish Giant). June 19,1919. Sick six weeks. Thick, white discharge. Cultures from nares. Numerous colonies of small bacillus; B. bronchisepticus in pure culture.

7. Private stock (young suckling, Flemish Giant). June 19, 1919. Nursing sick mother; sneezing. Killed and posted. Cultures from nares, blood, trachea and nasal sinuses. Numerous colonies in large numbers; B. bronchisepticus in nares and trachea in pure culture; Bact. lepisepticum in sinus.

10. Laboratory rabbit. June 18, 1919. Length of illness not known; thick purulent discharge. Killed and posted. Cultures from nares, trachea and blood. Several unidentified organisms in nares only.

11. Laboratory rabbit. June 18, 1919. History not known; thick purulent discharge. Killed and posted. Cultures from nares, trachea and blood. Bact. lepisepticum in blood; unidentified organisms from nares.

12. Laboratory rabbit. June 18, 1919. History not known; thick discharge. Killed and posted. Cultures from nares, trachea and blood. Bact. lepisepticum in blood; unidentified organisms from nares.

13. Laboratory rabbit. June 18, 1919. History unknown; watery discharge. Killed and posted. Cultures from nares, trachea and blood. Unidentified organisms from nares only.

14. Laboratory rabbit. June 18, 1919. History unknown; thick purulent discharge. Killed and posted. Cultures from nares, trachea and blood. Unidentified organisms from nares only.

15. Laboratory rabbit. June 18, 1919. History unknown; thin watery discharge. Killed and posted. Cultures from nares, trachea and blood. Unidentified organisms from nares; Bact. lepisepticum from blood.

17. Laboratory rabbit. June 19, 1919. History unknown; watery discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Bact. lepisepticum and B. coli from nares; B. bronchisepticus and B. coli from trachea.

18. Laboratory rabbit. June 19, 1919. History unknown; slight discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Unidentified organisms from nares; Bact. lepisepticum from sinus.

19. Laboratory rabbit. June 20, 1919. History unknown; watery discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Bact. lepisepticum from sinus.

20. Laboratory rabbit. June 20, 1919. History unknown; slight discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Unidentified organisms from nares; Bact. lepisepticum from trachea and sinus.

21. Laboratory rabbit. June 18, 1919. History unknown; thick purulent discharge. Cultures from nares. Organisms unidentified.

22. Laboratory rabbit. June 18, 1919. History unknown; thin watery discharge. Cultures taken from nares. Organism unidentified.

23. Laboratory rabbit. June 30, 1919. History unknown; slight watery discharge. Killed and posted. Cultures from nares, trachea, sinus, blood, spleen and kidney. B. bronchisepticus in large numbers in nares and also in pure culture in the trachea and sinus.

24. Laboratory rabbit. June 30, 1919. History unknown; slight discharge. Killed and posted. Cultures from nares, trachea, sinus, blood, spleen, liver and kidney. B. bronchisepticus from trachea and sinus in pure cultures.

25. Laboratory rabbit. June 30, 1919. History unknown; sneezing; no discharge. Killed and posted. Cultures from nares, trachea, sinus and blood. Unidentified organism from nares.

31. Private stock (2 months old doe, Flemish Giant). Aug. 11, 1919. Ill four days; dead six hours; slight watery discharge. Posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from sinus; B. bronchisepticus with other organism from nares.

32. Private stock (young doe, Flemish Giant). Aug. 11, 1919. Sick few days; slight watery discharge and sneezing. Killed and posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from both situations.

33. Private stock (young doe, Flemish Giant). Aug. 11, 1919. Sick five days; slight watery discharge. Killed and posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from sinus.

34. Private stock (buck, fourteen months old, Flemish Giant). Aug. 12, 1919. Sick six months; thick white discharge. Cultures from nares. Staph. albus; B. coli and other gas producers.

35. Private stock (young doe, common rabbit, wet nurse). Aug. 12, 1919. Sick few days; slight watery discharge. Killed and posted. Cultures taken from sinus. B. bronchisepticus in pure culture.

36. Private stock (young wet nurse, common rabbit). Aug. 12, 1919. Sick one week. thick discharge. Killed and posted. Cultures from nares and sinus. B. bronchisepticus from sinus in pure culture; B. bronchisepticus associated with other organisms from nares.

37. Private stock (11 months old doe, Rufus Red). Aug. 12, 1919. Sick three weeks; thick white discharge. Killed and posted. Cultures from nares and sinus. B. bronchisepticus in pure culture from both places.

43. Private stock (young doe, New Zealand Red). Aug. 13, 1919. Symptoms one week; slight watery discharge. Killed and posted. Cultures from sinus. B. bronchisepticus in pure culture.

44. Private stock (young doe, New Zealand Red). Aug. 13, 1919. Sick few days; slight discharge. Killed and posted. Cultures from sinus. B. bronchisepticus.

46. Private stock (doe, with young, New Zealand Red). Aug. 13, 1919. Sick few days. Young rabbits perfectly healthy. Cultures from nares. Staph. albus.

50. Private stock (young rabbit, Flemish Giant). Well when brought in Aug. 13, 1919; rubbing nose, Aug. 16, 1919; dead Aug 18, 1919. Cultures from sinus. Unidentified organism.

51. Private stock (young rabbit, Flemish Giant). Sick when brought in Aug. 13, 1919; discharge from nose, Aug. 15, 1919; sneezing and very sick, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Bact. lepisepticum in sinus.

52. Private stock (young rabbit, Flemish Giant). Slight symptoms when brought in Aug. 13, 1919; considerable discharge, Aug. 15, 1919; very sick, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Bact. lepisepticum in sinus.

53. Private stock (young rabbit, Flemish Giant). Well when first seen, Aug. 13, 1919; no symptoms, Aug. 16, 1919; killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Bact. lepisepticum in sinus.

54. Private stock (young rabbit, Flemish Giant). Slight symptoms, Aug. 13, 1919; rubbing nose, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus. Staph. albus.

55. Private stock (young rabbit, Flemish Giant). Well when first seen, Aug. 13, 1919; slight symptoms Aug. 15, 1919; nose moist, Aug. 16, 1919. Killed and posted, Aug. 18, 1919. Cultures from sinus and blood. Unidentified.

60. Private stock. (Flemish Giant.) Chronic case; thick discharge. Cultures from nares. Gas producer and Staph. aureus.

61. Private stock (Flemish Giant). Chronic case; thick purulent discharge. Cultures from nares. Gas producer and B. bronchisepticus.

Total rabbits examined—40.

CONTROL OF THE DISEASE

If the control of snuffles is to be attempted it must be handled as any contagious disease of bacterial origin; namely, by the observance of the ordinary sani-

tary precautions and hygienic measures and the use of specific therapy wherever applicable.

In 1914 one of us (N. S. F.)[8] reported the control of a severe local outbreak of an infection with B. bronchisepticus in rabbits by means of prophylactic inoculations with a specific vaccine containing 100 million bacteria per cubic centimeter. Each animal received the vaccine every third day, starting with one cubic centimeter and doubling the dose at each subsequent injection until three injections had been given. At the same time the author reported the unsuccessful attempt to control an infection due to a bacillus of the hemorrhagic septicemia type; later experiments, however, have taught us that the dose was probably much too small. The most recent work of the authors has shown that a larger dose of both organisms may be given with more satisfactory results; in fact, they recommend the use of a vaccine containing 400 million each of B. bronchisepticus and Bact. lepisepticum and 200 Staph. albus as the initial dose.

According to reports from various localities relative to the use of this vaccine it would appear that about 90 per cent of the rabbits were protected after prophylactic inoculation, and of those cases where treatment was instituted after the disease was well established, about 50 per cent were relieved of symptoms.

DISCUSSION

From the results of the present as well as former investigations, it is very evident that snuffles in rabbits is not a single entity, due to any one microorganism, but is more or less of a symptom following an infection of the upper respiratory tract with any one or more of several organisms. To further substantiate this statement, in addition to the findings of the authors already quoted, Ward[13] experimentally produced snuffles in rabbits by the intravenous injections of B. ozenæ, B. proteus and B. bronchisepticus.

It seems to be evident that the ordinary form of snuffles as encountered in the various rabbitries in this country, characterized by a variable nasal discharge accompanied by sneezing and rubbing the nose, with more or less loss of appetite and weight and a rather subacute or chronic course (which constitutes the large majority of cases), is caused by B. bronchisepticus; while the more acute form and the most fatal, in the majority of instances is due to Bact. lepisepticum. This statement is not based on absolute findings, as both organisms have been encountered in various types of cases, but is the opinion of the authors, judging from the well recognized pathogenic powers of the two organisms and a general picture of all the cases they have observed taken as a whole.

It seems to be a fact, also, from a careful perusal of the literature relating to infections of the respiratory tract of rabbits, that these conditions are becoming less severe and are changing from acute general infections, with the most prominent symptoms associated with the lungs and surrounding tissues to a more subacute and chronic condition, with symptoms referring to the upper respiratory tract only. Formerly all the epizootics seemed to be sporadic in nature and extremely severe, while at the present time the infection appears to be constantly with us, spreading over the entire country, now and then increasing in severity in certain localities.

In this connection it might be stated that the opinion is rather general among those who have examined available literature on the subject of snuffles, that it is a form of hemorrhagic septicemia. This no doubt is explained for the reason that veterinary textbooks give a prominent place to the rabbit septicemia organism in diseases of rabbits. This is probably due to the tendency of previous workers to investigate acute diseases of these animals, and give less attention to a chronic nonfatal disease as is characteristic of snuffles at the present time.

The fact should be kept in mind that certain members of the hemorrhagic septicemia group of organisms have been isolated from the respiratory tract of apparently healthy animals, and for this reason these organisms are classed with others such as the pneumococcus, for example, which is believed to become pathogenic under conditions which tend to lower the resistance of the host. The work of Davis, previously referred to, has shown that the "carrier" state in rabbits, as concerns the hemorrhagic septicemia organisms, is a condition to be reckoned with. One of the present writers (H. P. H.)[14] has called attention to the liability to obtain misleading results from rabbit inoculations, on account of the fact that the rabbits may actually be harboring the organisms at the time they are inoculated with suspected material for diagnostic purposes. In the event of the death of the rabbit, and obtaining a pure culture at autopsy, there is no way of determining definitely the actual source of the organism. The conditions under which hemorrhagic septicemia organisms are transformed from saprophytes to pathogens are probably quite varied, and in the disease being discussed in some cases, at least, we may have a double infection to deal with, probably a bronchisepticus infection supervening upon a previous hemorrhagic septicemia invasion, or vice versa.

The organism found most frequently as a secondary invader, and which probably is responsible for the purulent condition of the nasal discharge and perhaps for the chronicity of many of the cases, was Staph. albus. This organism was observed in almost every case.

Oddly enough the streptococcus, which is usually considered a common secondary invader in all infectious processes, especially of the respiratory tract, was not present in one of our cases. We must conclude, therefore, that the streptococcus is infrequently harbored by the rabbit and is not a factor to be reckoned with when undertaking to control diseases of this animal.

CONCLUSIONS

1. Contagious nasal catarrh of rabbits, commonly called "snuffles," may be caused by any one of several microorganisms.

2. B. bronchisepticus is responsible for the majority of cases of the ordinary snuffles encountered in this country at the present time.

3. Bact. lepisepticum is an important etiologic factor, as it was found in a large number of cases, and is probably responsible for many of the acute types of the disease which result fatally.

4. Staph. albus, probably present in the nature of a secondary invader, was found in practically all cases with purulent nasal discharges.

5. Many other organisms, especially gas producers of the B. coli type, were isolated but in such small numbers as to preclude their relationship to snuffles from an etiologic standpoint.

6. The streptococcus is probably not a factor to be considered in infections of the respiratory tract of the rabbit, as it was not found in a single case.

7. The disease can, in a large measure, be controlled with a vaccine composed of the three most prominent microorganisms mentioned above, especially if the conditions surrounding the animals are at all sanitary.

BIBLIOGRAPHY

[1]Beck: Ztschr. f. Hyg., 1893, xv, 363.
[2]Kraus: Ztschr. f. Hyg., 1897, xxiv, 396.
[3]Volk: Centralbl. f. Bakteriol., 1902, xxxi, 177.
[4]Südmersen: Centralbl. f. Bakteriol., 1905, xxxviii, 343.
[5]Ferry: Am. Vet. Rev., 1910, xxxvii, 499.
[6]Ferry: Jour. Infect. Dis., 1911, viii, 399.
[7]Ferry: Am. Vet. Rev., 1912, xli, 77.
[8]Ferry: The Vet. Jour., 1912, lxviii, 376.
[9]Ferry: Jour. Path. and Bacteriol., 1914, xviii, 445.
[10]M'Gowan: Jour. Path. and Bacteriol., 1911, xv, 372.
[11]Davis: Jour. Infect. Dis., 1913, xii, 42.
[12]Davis: Jour. Infect. Dis., 1917, xxi, 314.
[13]Ward: Jour. Infect. Dis., 1916, xix, 153.
[14]Hoskins: 22nd. Ann. Rpt. U. S. Live Stock Sanitary Assn., 1918, p. 123.

THE QUESTION OF THE TOXICITY OF LUNG EXTRACTS

By Plinn Morse, M.D., Detroit, Mich.

MOST laboratory workers, sooner or later in their experimental work, encounter the fact that lung extracts are highly poisonous when given rapidly by the intravenous route. This result without proper controls has undoubtedly led many into errors in interpreting their results. Some years ago, in trying to prove a tissue specificity of lung tissue for the tubercle bacillus, V. C. Vaughan, Jr., and the writer encountered this phenomenon, and for a while gave it some attention under the impression that it constituted an original observation. In association with Miss Stott the subject was pursued further, and lung tissue was found more toxic than that of other organs, but the extract of scrapings from the intima of the aorta was far more potent than any organ extract examined. The poison did not pass a Berkefeld filter and did not dialyse, and the conclusion was reached that it was apparently related to the phospholipin content of the tissue. Heating to 70 degrees destroyed its toxic properties, but heating to 56 degrees had no effect. We always found the poison confined with the nucleoprotein fraction of the protein precipitate, but thymus nucleic acid made by ourselves, and yeast nucleic acid purchased from Merck did not contain the poison. The mode of death was suggestive of anaphylaxis, but postmortem quickly showed that death was due to massive coagulation of the venous blood in the large veins and right heart. The conclusion was arrived at that the extract of endothelial cells contained a powerful activator of coagulation which was probably identical with that present in leucocytes and might be classed with those substances loosely called thrombokinase. We found by referring to the literature that all these facts were well known and that a considerable bibliography already existed covering all the points we had determined and many others in addition.

The effect of tissue extract as a coagulin led Loeb to give the name "Tissue Coagulins" to these substances and the literature of the subject is closely bound up with that of coagulation of the blood. It is the writer's impression, from inquiring among laboratory workers, that the lethal action of lung extract due to the "Tissue Coagulin" content is quite well known to those who have worked along the lines of experimental pathology. Nevertheless, in the last fifteen years very little has been written about this particular phenomenon and textbooks have no occasion to call it to the attention of students.

Several articles have appeared recently which have revived the interest in lethal organ extracts. Wherry and Ervin have called attention to the toxicity of lung extracts and publish their findings as original observations without a bibliography. From the context of the article one gathers the impression that they believed they were dealing with a hitherto unobserved phenomenon. They did not determine that death was due to intravascular clotting, but did find that the blood found in the vessels at autopsy had a delayed clotting time, a fact that is clearly stated in the older literature.

Wherry and Ervin noted that though in many ways the manner of death resembled that due to anaphylaxis, there were important differences in that the lungs collapsed on opening the thorax of lung-extract killed animals and that atropin does not protect. They also found, again confirming earlier work, that a sublethal dose of organ extract protected against a lethal dose of lung extract given an hour later. This "negative phase" of coagulability is a well-known phenomenon following organ extract injections.

Later Kowsaku Kakinuma studied the toxicity of lung extracts. Kakinuma was apparently well aware that the subject was not new and appends a partial bibliography of the previous work on this subject. He showed that glucose and adrenalin had each a certain power to protect against the lethal action of the lung extract and that the blood sugar of an animal was raised after a sublethal dose.

Kakinuma gives Dold (1911) the credit for observing that intravenous administration of extract of normal lung killed animals, but this phenomenon was observed before this and is referred to by Loeb.

The most exhaustive articles on the subject that have come to the writer's attention are those of Loeb (1904) (1907) the latter with a very complete bibliography. Still more recently C. A. Mills, believing that Wherry and Ervin had been the first to observe this phenomenon studied the question further and determined that lung tissue was more toxic than that of other organs, although Loeb had already shown that an extract of the scrapings of the aortic intima was the most toxic of all. Mills rediscovered the fact that death was due to intravascular clotting.

The articles of Wherry and Ervin and of Mills have been of service in that they have called attention in the recent literature to a fact that seems to have disappeared from current printed pages in the last few years.

A partial bibliography, including the more important of the older articles, is appended. Very complete bibliographies are given by Loeb and Morawitz, and Kakinuma mentions several important articles.

BIBLIOGRAPHY

Aronson: Berl. klin. Wchnschr., 1913, No. 6.
Dold: Ztschr. f. Immunitatsf., x (see also bibliography by Kakinuma).
Doyon: Arch. Path. and Physiol., 1912, xiv, 229.
Gutman, L. H.: Ueber die Blutveranderungen bei der Vergiftung mit Organextrakten, Ztschr. f. Immunitatsf., 1913, No. 4, pp. 362, 372.
Howell: Am. Jour. Physiol., 1911, xxix, 187.
Izar Palane: Ztschr. f. Immunitatsf., xiv.
Kakinuma, Kowsaku: Am. Jour. Physiol., I, 9.
Kuster, Hermann: Ergebn. d. im Med. u. Kinderheilk., 1913, xii, 666, 732.
Loeb, Leo.: Biochem. Centralbl., 1907, iv, 829; also Bull. Univ. of Penn. Med., 1904, xvi, 382.
Mills, C. A.: Jour. Biol. Chem., vi, 425.
Morawitz: Ergebn. d. Physiol., abt. 1, 1904, iv, 307; also Handbuch d. Biochem., 1908, ii, 40.
Muraschew: Deutsch. Arch. klin. Med., 1904, lxxx, 187.
Wherry and Ervin: Jour. Infect. Dis., xxiii, 240.
Wohlgemuth: Biochem. Ztschr. xxv.

A SIMPLIFIED METHOD FOR THE DETECTION AND ESTIMATION OF THE DISTRIBUTION OF MORPHINE*

By Sergius Morgulis and Victor E. Levine, Omaha, Nebr.

THE observations here recorded arose out of an attempt to simplify the procedure of testing for the presence of morphine, which we applied to a sample of poisoned meat sent to our laboratory for investigation. As a result of our effort a method has been worked out which can be easily employed in a clinical laboratory for the detection of morphine in food, body tissue or fluids.

The specimen under examination is finely minced and heated on the water-bath for 30 minutes with 100 c.c. of a 2 per cent aqueous tartaric acid solution for every 20 gm. of substance. The morphine, when present, is converted to soluble morphine tartrate. The materiai is cooled, preferably in the ice chest to facilitate the solidification of all the fatty matter. The material is then strained through cheesecloth which retains most of the solid particles, and washed until the washings are no longer acid. The liquid portion upon refiltering through paper gives a clear, usually somewhat opalescent fluid. The filtrate is evaporated to a thick consistency and sodium bicarbonate is added in small amounts until effervescence ceases; after which a small excess is added. The morphine tartrate is thus decomposed and the alkaloid set free. The pasty mass is now evaporated to dryness, ground to a fine powder i an mortar and extracted with chloroform.

In order to establish the sensitiveness of the reaction, an aqueous solution of morphine sulphate was prepared, 1 c.c. of which corresponded to 0.01 mg. morphine. Definite volumes of this solution were evaporated on the water bath in a porcelain crucible and after cooling, five to ten drops of a freshly prepared selenious-sulphuric acid reagent (0.5 gm. selenium dioxide in 100 c.c. concentrated sulphuric acid) were added to the residue. We found that 0.01 mg. morphine equivalent to 0.025 mg. morphine sulphate yielded a positive reaction, while 0.005 mg. morphine corresponding to 0.0125 mg. morphine sulphate gave a faint but recognizable positive test. Selenium dissolves in concentrated sulphuric acid and forms selenosulphur trioxide, which gives a green solution. It is probable that reduction of selenious acid to free selenium through the reducing action of morphine occurs, leading to the production of a green color, owing to the interaction of the selenium and the sulphuric acid. In the presence of moisture the selenosulphur trioxide is decomposed, giving brick-red selenium which may remain in colloidal form or which may precipitate out. The color developed in the test with morphine disappears on standing, heating, or on adding water; it is replaced by a reddish brown color.

To determine how much morphine can be recovered when mixed with biologic material two experiments were performed with meat and one with liver. Twelve portions of 20 gm. each of the finely minced material, containing respectively (1) 0.00 mg.; (2) 0.005 mg.; (3) 0.01 mg.; (4) 0.05 mg.; (5) 0.1 mg.; (6) 0.2 mg.; (7) 0.3 mg.; (8) 0.4 mg.; (9) 0.5 mg.; (10) 0.6 mg.; (11) 0.7 mg.; (12) 1 mg. were heated on the water-bath with 100 c.c. 2 per cent

*From the Biochemical Laboratory, Creighton University, College of Medicine, Omaha, Nebr.

tartaric acid for one-half hour and examined for morphine, in the manner already described using 50 c.c. chloroform for extraction. No less than 0.2 mg. morphine equivalent to 0.5 mg. morphine sulphate could be detected.

The first one to report a selenium compound as an alkaloidal reagent was Brandt, who, in 1875, made use of concentrated sulphuric acid containing selenic acid for the detection of morphine and narcotine. Morphine gave at first a bluish green, then grass-green, which quickly changed into reddish yellow and reddish brown. Narcotine first produced a blue color, which became green and, finally, also changed to reddish yellow or reddish brown. The limit of reaction for morphine he gave as 1/5 to 1/6 mg. and for narcotine as 1/10 mg. In 1885 Lafon used ammonium selenite in concentrated sulphuric acid. He obtained green colorations with codeine or morphine. The limit of sensitiveness for codeine was reported as 0.1 mg. In 1891 Ferreia da Silva obtained the characteristic reaction with morphine, codeine, narceine, and narcotine. In 1899 Mecke described experiments with an alkaloidal reagent consisting of 0.5 gm. selenious acid in concentrated sulphuric acid. He highly recommended its use in detecting the opium alkaloids. Morphine gave an evanescent blue, then intense blue, then green to persistent olive green; codeine, blue quickly changing to emerald green, and later to persistent olive; narceine, faint greenish yellow, then violet; narcotine, greenish steel blue, later cherry red; papaverine, greenish, dark steel blue, then deep violet resembling methyl violet; thebaine, deep orange, gradually paling; apomorphine dark blue, violet. Mecke made a number of comparative tests with the selenium reagent, and with several others. He found the selenium test more sensitive than the Pellagri test, nitric acid test, and Froehde's test. The characteristic color developed with the selenium appears in the cold. In practical work which involves the testing of alkaloids in tissues, the heat necessary for the extraction process gives rise to brown impurities, which often hide the color produced by alkaloidal reagents. The characteristic selenium reaction is apparent even under such conditions, provided no water is present.

The selenium reagent, sometimes called Lafon's reagent, or Mecke's reagent, gives a characteristic bluish or olive green coloration with the opium alkaloids mentioned, except thebaine, which gives a deep orange gradually paling. We have also found heroine and dionine to give the bluish olive green reaction and Lafon observed that oxydimorphine gives a brownish violet color changing to violet. On account of its sensitiveness and specificity for the opium alkaloids the reagent should be a very useful indication for them.

Several animal experiments were performed with the idea of determining the distribution of morphine as identified by the selenious-sulphuric acid reagent, which has been but recently introduced into toxicology. Our experiments represent acute morphine intoxication so that our findings would be of value from a forensic point of view as the deaths usually reported from morphine poisoning are those of the acute type. The only record we have been able to find on the use of the selenious-sulphuric acid reagent in the literature, was in the case of a Portuguese physician (1891-3) who was accused of having poisoned three of his wife's nephews, and of having brought about the death of one of them by poison

given in enemata. The symptoms were those caused by opium in part only. Morphine, narcotine and delphinin were found in the urine and in the viscera. The presence of morphine was revealed by the iodic test, Froehde's and the selenious-sulphuric acid reagents.

To determine the distribution of morphine in the animal organism, we adopted the following procedure. The organs were weighed and treated according to the method already outlined and of the final chloroform extract the smallest quantity was found, which, when evaporated, gave a positive reaction with the selenious-sulphuric acid reagent. The amount of morphine in the total extract was obtained in the following calculation:

$$\frac{V \times 0.01}{v} = \text{Mg. Morphine in sample.}$$

V = the volume of the total extract; v = volume of extract giving a positive reaction. The smallest amount of morphine that can be detected with certainty is 0.01 mg.

EXPERIMENT No. 1, MAY 27, 1919.*

May 10. Male rabbit, 1.5 kg. subcutaneous injection of 450 mg. morphine sulphate.
May 27. injected 800 mg. of morphine; found dead next morning.

	ORGAN	WEIGHT OF ORGAN IN GRAMS	RELATIVE MORPHINE CONTENT
1	Liver	66	100
2	Bladder and urine	3.5	91
3	Lungs	16.8	57
4	Kidneys	18.9	19
5	Brain	8.0	14
6	Heart	7.7	4
7	Spleen	1.0	3
8	Stomach* and contents	121.5	0.5
9	Pancreas	7.0	trace
10	Small int. and contents	72.0	—
11	Large int. and contents	44.0	—
12	Cecum and contents	120.2	—

*The chloroform extracts of stomach, small and large intestines and cecum were greenish owing to the chlorophyl of the ingested food. On evaporation a greenish residue was left. Since it was impossible to apply the selenium reagent to the colored residues, these were extracted with ether, in which morphine is insoluble. The ether extracts were filtered through paper, and again dissolved in chloroform. On evaporating this second chloroform extract, negative reactions were obtained with small and large intestines and cecum, while the stomach yielded a slight positive test.

The trend of opinion is that morphine is eliminated principally by the alimentary canal. Alt first demonstrated its elimination by the stomach and Vogt first detected morphine in the stools of a morphinist. The latter found on washing the stomach after hypodermic injection of morphine about one-half the amount recoverable in that organ, and that the elimination began in from one to three minutes and ceased completely in from fifty to sixty minutes. Hamburger reported both morphine and meconic acid in the stomach washings in the case of opium poisoning *per os.* Binz in experiments on men found morphine in the stomach contents two and a half minutes after the hypodermic injection of 0.03 gm. morphine. Tauber, with dogs, recovered after hypodermic injection 41.3 per cent in the feces; and Faust in similar experiments recovered 70.9 per cent while 62.2 per cent morphine has also been detected in the feces after hypodermic administration. In experiments with rabbits Neuman and Tetze failed to obtain

EXPERIMENT NO. 2, JUNE 23, 1919

Rabbit, 2.1 Kg. given by mouth one gram morphine sulphate.

	ORGAN	WEIGHT OF ORGAN IN GRMS	RELATIVE MORPHINE CONTENT
1	Stomach and contents	94.	100
2	Urine	(28 cc)	64
3	Small int. and contents	74.5	12
4	Cecum and contents	109.0	8
5	Large int. and contents	48.5	4
6	Lungs	19.0	2
7	Liver	53.0	1.5
8	Brain	8.5	1.0
9	Kidneys	15.2	0.9
10	Blood	9.5	0.6
11	Heart	7.5	0.3
12	Pancreas	3.5	0.23
13	Urinary bladder	1.5	0.19
14	Spleen	1.5	0.17
15	Gall bladder	0.5	0.03
16	Muscle	52.0	

so large a proportion in the feces as those found by Tauber and Faust with dogs, probably because of the greater reabsorption in the large intestines of rabbits.

It is evident from laboratory findings that morphine, however administered, will most probably be detected in the alimentary canal in the material removed from the stomach by vomiting or by lavage. Analyses performed for medico-legal purposes have very frequently failed to demonstrate the presence of morphine in well marked and known cases of opiate poisoning even when made soon after the ingestion and under conditions apparently favorable. Witthaus cites very many cases where morphine could not at all be detected in the stomach. It does not seem that the pathway of elimination or that the distribution follows any regular scheme.

In a number of undoubted opiate poisonings as well as in cases of morphinists taking large quantities, analyses of the urine have failed to show the presence of morphine. In others, however, morphine has been demonstrated repeatedly in the urine. Bischoff in some of his postmortems found nothing in the stomach and but traces in the urine. Lesser reported morphine in the urine, but nowhere else in the cadaver. It is believed that renal elimination plays a secondary part to that of the alimentary canal and with small doses may be inoperative. Our experiments indicate that renal elimination of morphine is probably of no less importance than elimination through the alimentary canal, especially in acute poisoning, and that from a toxicologic standpoint the examination of urine is essential.

Marcelet in his study of the localization of morphine in different portions of the organism of an addict records the following relative distribution:

Liver + + + +
Stomach + + +
Kidney + + +
Heart +
Brain +
Lungs +

These results compare well with our experiment on the rabbit which received morphine by subcutaneous injection. Marcelet's addict was doubtless also taking morphine hypodermically. In both instances the liver is found to contain the largest amount of the alkaloid, while the kidneys (and urine) contain only somewhat less than the liver. These independent analyses suggest the probability that in morphine poisoning the liver assumes an important part if the morphine is given as a subcutaneous injection. From a forensic point of view these experimental findings are significant in that they emphasize the necessity of determining the relative amounts of morphine in alimentary canal, liver, kidney and urine.

The fact that morphine is not always detected in the urine has led to the supposition that the alkaloid is modified and rendered undetectable in the system. Rubsamen has shown that certain proportions of injected morphine disappear in the bodies of rats and that these proportions become larger by habituation. The disappearance is ascribed to oxidation or conjugation. Marme reported in urine a substance which differed from morphine in giving a nontypical test with the Husemann and Froehde reagents. He believed this substance to be identical with the oxydimorphine obtained by oxidation *in vitro* but Donath and Stolnikow and also Marquis failed to confirm the finding. Marquis claimed morphine to be eliminated in part at least in the form of a conjugated compound. Although morphine through its phenolic hydroxyl has the power of forming ester sulphates similar to those normally present in the urine, monomorphyl sulphate is not found in the urine after administration of morphine. The proportion of ethereal sulphate in the urine, however, is nevertheless increased. The occurrence in urine after the administration of morphine of a reducing substance argued for a glycuronic pairing, but recently the experiments of Spitta have demonstrated that the carbohydrate component is not glycuronic acid but an unknown acid related to laiose. In our experiments the fact that large quantities of morphine were recovered in the urine probably indicates that the selenious-sulphuric acid can detect morphine even in a modified or paired condition.

Our experimental findings point to the precaution that in the chemical examination of a cadaver for morphine no portion of the body should be overlooked. Even the salivary secretion is of importance in this respect, for Rosenthal, in a patient receiving hypodermically therapeutic doses of morphine, found considerable quantities of the alkaloid in the saliva.

SUMMARY

Morphine can conveniently be determined in food, or in tissues and body fluids by heating with 2 per cent tartaric acid (if solid, the material should first be ground or finely minced) to convert all morphine into the soluble tartrate. The mixture is rapidly cooled, preferably on ice, to solidify the fatty material. The solid residue is removed by straining through cheese cloth, and washed until the washings are no longer acid to litmus. The liquid, after being filtered through paper, is evaporated to a pasty consistency. The tartrate is then decomposed by the addition of an excess of solid sodium bicarbonate which sets the alkaloid free. The evaporation is then continued to complete dryness, the mass

powdered and extracted with chloroform to remove the free morphine. The volume of the chloroform extract is noted, and the smallest quantity of the extract is found which on evaporation (in a porcelain crucible over the water-bath) leaves a residue which yields a definite morphine test. In this way the relative amount of morphine in several extracts can be determined; besides, knowing the limit of sensitivity of the reaction an approximate estimate of the amount of morphine in the original sample is possible.

The various alkaloidal tests can be applied to the residues after the evaporation of portions of the chloroform extract. The reagent we employed, selenium dioxide dissolved in concentrated sulphuric acid, is very sensitive towards the opium alkaloids. While the limit of sensitivity for morphine may be regarded as 0.005 mg. yet for practical purposes the smallest amount that can be unmistakably identified as morphine with this reagent is 0.01 mg.

We conclude from our experiments with rabbits that morphine whether given subcutaneously or by mouth is widely distributed throughout the animal body, finding its way into almost every tissue. The morphine is invariably found in appreciable quantities in the urine and kidney. Also large quantities may be present in the alimentary tract, liver, lungs and brain. According to our results especially large amounts of morphine were present in the alimentary tract and excretory organs after administering the poison by mouth, while after injecting under the skin it was recovered principally from the liver, excretory organs, and also from the lungs and brain.

We conclude furthermore, that it is not advisable to limit the toxicologic examination for morphine to the alimentary tract alone, an examination of at least the kidney and urine, and liver being indispensable.

BIBLIOGRAPHY

Autenrieth, W.: Detection of Poisons, translated by W. H. Warren, ed. 4. p. 108.

Brandt, C.: Ueber eine neue Alkaloidreaction mit Selen-und Tellursäure, Dissertation, Rostock, 1875; Jahresb. ü. d. Pharm., 1875, p. 341.

Ferreira da Silva, A. J.: Sur l'emploi du sulfo-séléníde d'ammoniaque poui characteriser les alcaloides, Jour. de pharm. et de chim., 1891, xxiv, 102.

Lafon, P. L.: Action des séléniates et des sélénites sur les alkaloides, Compt. rend. Acad. sc., 1885, c, 1543.

Mecke: Ein neues Reagens auf Alkaloide, Nachweis von Opium, Ztschr. f. öffentliche Chemie, 1899, v, 351.

Marcelet, H.: Localization of Morphine in the Human Body, Bull. sc. pharm., 1918, xxv, 292.

Witthaus, R. A.: Medical Jurisprudence, Forensic Medicine and Toxicology, New York, W. Wood & Co., iv.

LABORATORY METHODS

WASSERMANN RESULTS WITH ANTICOMPLEMENTARY SERA

By Miles J. Breuer, M.A., M.D., Lincoln, Nebr.

WHEN a serum turns out to be anticomplementary, as a rule no other thought occurs to the serologist than to discard it and request another specimen. I had never given the matter any other thought until the following case was met with, in doing serologic work at a Base Hospital Center in France:

A specimen was sent into the laboratory by the medical officer in charge of the genitourinary ward, and was found to be anticomplementary. The serum was perfectly clear, about 24 hours old when the test was made, and had been kept cold. Another specimen was requested, in the routine manner, and was again found to be anticomplementary. This time I saw the ward surgeon personally, and requested that all possible precautions be taken in obtaining the third specimen. This was taken at once into the laboratory, and the serum separated immediately after clotting had taken place; and the test run on the same day. The serum was again anticomplementary. For some reason this patient's serum possessed the property of absorbing complement; specimens taken a month and two months later were identical in the possession of this property.

As it was very desirable to get a Wassermann result on this man, the complement-fixing power of the serum was titrated as follows: A series of ten tubes was set up, containing 1 c.c. of normal salt solution, 0.1 c.c. of the patient's serum, and quantities of complement from 0.1 c.c. to 1.0 c.c. in steps of 0.1 c.c. This was incubated for an hour, and then amboceptor and cells added, and the incubation continued for 30 minutes. The first tube showing complete hemolysis was the seventh. The dose of complement being 0.4 c.c. the anticomplementary power of the serum was represented by 0.3 c.c. of the complement. The 0.7 c.c. represented the dose of complement required in the reaction, including the excess over the normal dose represented by the serum's anticomplementary power. With this amount of complement a satisfactory double-plus Wassermann result was obtained, differing in no way from the results with the rest of the sera. Consistent results were obtained upon repeating the test a month and two months later. No Wassermann result could have been obtained on this patient without this procedure.

Upon resuming serologic work in civil practice, this method suggested itself when anticomplementary results were obtained on sera sent into the laboratory by physicians in general practice. In military work, or in a large hospital, when a serum is found anticomplementary, it is a small matter to get another specimen. In private practice there is a good deal of hesitation in asking patients to allow

themselves to be punctured a second time; the procedure is none too popular. In case of sera shipped from a distance, the matter of delay also enters. As a rule, the delay makes little difference in the actual course of the disease, but makes a great deal of difference in the mental attitude of the patient. There are very few people who will not become difficult to handle as private patients, if they have to wait a week for a report, and then instead of getting one, find they have to have the needle in their arm a second time.

The titration of the anticomplementary power of serum was therefore tried out in seven cases of sera which were found to be anticomplementary. The results are here shown:

SERUM	ANTICOMPLEMENTARY POWER	DOSE OF COMPLEMENT	SPECIMEN HEMOLYZED	APPROXIMATE AGE OF SPECIMEN	RESULT
1	0.7 c.c.	0.5 c.c.	yes	4 days	+ +
2	0.4 c.c.	0.6 c.c.	yes	4 days	+ +
3	0.4 c.c.	0.5 c.c.	yes	6 days	+
4	0.2 c.c.	0.4 c.c.	no	24 hours	neg
5	1.0 c.c.	0.6 c.c.	yes	7 days	+ +
6	0.3 c.c.	0.5 c.c.	yes	3 days	+
7	0.4 c.c.	0.5 c.c.	yes	4 days	neg

In all of these cases, the results were clear cut, with the exception of the fifth. In this one, the control tube was not clearly hemolyzed, and it was considered advisable to report that another trial should be made. In all of these cases, the anticomplementary action was clearly due to deterioration or poor preservation, with the possible exception of No. 4. While the number of observations in this preliminary report is too small to warrant any definite conclusions, the following points are suggested: That a high anticomplementary power does not interfere with a clear-cut positive reaction, and therefore probably the syphilitic amboceptor is not concerned in the absorption of the complement occurring in anticomplementary action; that the anticomplementary action depends on hemolysis in most cases, and increases with the age of the specimen.

The question of whether or not it is practical to undertake this titration and repetition of the test after the main portion of the specimen has been run, in preference to getting another specimen, must be left with the individual worker.

BARRON'S POLYCHROME TOLUIDIN BLUE AS A SUBSTITUTE FOR METHYLENE BLUE IN THE EOSIN-METHYLENE BLUE METHOD OF MALLORY*

By R. C. Whitman, M.D., Boulder, Colo.

SHORTLY after Barron published in this journal, (1918, iii, 432) his method for polychroming toluidin blue I began using his solution for staining sections cut frozen for rapid diagnosis. The results were so satisfactory that I soon found myself using the same solution as a routine substitute for alkaline methylene blue, and I have now been employing it for more than a year in this way, particularly in the eosin-methylene blue method of Mallory. I have been so pleased with the results obtained that I give the method herewith.

The toluidin blue solution is prepared by Barron's method as follows: Dissolve toluidin blue, 1, and potassium carbonate, 1, in distilled water, 400. Boil in a glass beaker to 300. Cool and add toluidin blue 2, sodium chloride 3, glacial acetic acid 12, and alcohol 15. It is ready for use at once, and in my experience seems to improve with age.

The staining method is:

1. Fixation in Zenker's, Orth's or Helly's fluid. Formalin is quite unsuitable.
2. Celloidin or paraffin embedding.
3. Fairly strong yellowish water soluble eosin. (I use 5 per cent), five or ten minutes.
4. Water.
5. The above toluidin blue solution, diluted 5, 10, or 20 times with water, for five or ten minutes, or until the nuclei are stained about as deeply, or only slightly more deeply than it is desired they should appear in the finished preparation. The dilute solution keeps indefinitely.
6. Water.
7. 95 per cent alcohol to differentiate.

8a. Paraffin sections, absolute alcohol, xylol, balsam.

8b. Celloidin sections, clearing oil (I use Dunham's mixture), slide, balsam.

The following points of difference with Mallory's method may be noted:

a. Equally satisfactory results are obtained after any of the fixing fluids mentioned above, except formalin.

b. Differentiation is vastly less tricky. With paraffin sections overdifferentiation is almost impossible. Indeed, overstaining with the blue should be avoided. Celloidin sections seem to decolorize more readily, and therefore need to be watched more carefully.

c. The blue is not affected by clearing oil, (Dunham's), making the method more easily applicable to celloidin sections.

d. The toluidin blue is decidedly more opaque than methylene blue, and hence gives greater contrast in photographic plates.

The stain differentiates with extraordinary sharpness and brilliancy, and gives truly exquisite color gradations.

*From the Henry S. Denison Memorial Research Laboratories.

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EDITORIALS

The Transmissions of Respiratory Infection

IN A STUDY of those infectious diseases in which the portal of entry appears to be the respiratory tract, we may divide the infection cycle for convenience into a number of subdivisions or phases. By infection cycle we would designate all the phenomena that occur from the time a virus leaves the body of one individual until it leaves that of a second, after having been transmitted in some manner to the second, having been deposited on the skin or mucous membranes, having actually invaded the tissues, and finally having been liberated from the surface to begin again its infection cycle. In its broader sense these phenomena will include, not only the life history and peregrinations of the organism, but will include all of the reactions of the infected host. It is fortunate that much of the work undertaken as a result of the influenza epidemic will be of great value in the study of the pathogenesis of other so-called respiratory infections and, conversely, that even though we may now have little or no influenza, increased knowledge of the pathogenesis of these other conditions will facilitate the ultimate description of the infection cycle in this greatest of pandemic diseases.

The three phases in the consideration of the pathogenesis of respiratory infections as described by Bloomfield[1] are: first, the means whereby the virus is conveyed to the individual; second, the fate of the organism from the time it reaches the mouth or nose until it is eliminated or until invasion takes place; and third, the actual invasion of the virus into the body. It might even be well to subdivide the first phase into first, manner of leaving the originally infected individual, and second, history up until its deposition on the mucous membrane or other locality of the exposed individual. We possess much valuable information concerning each of the phases, but many links in the chain are missing in all.

Means Whereby the Virus is Conveyed to the Individual.—Three factors in the transfer of virus from the infected to the healthy are receiving chief emphasis at the present time. First among these is "droplet infection," then contamination of hands, clothing, etc., and finally, contamination of eating utensils. Droplet infection, a mode of spread which has been recognized for many years, has, following particularly the work of Weaver,[2] received great emphasis. It has resulted, especially in the army, in the adoption of masks and the cubicle system. Weaver was able on diphtheria wards to reduce the incidence of diphtheria carriers among the nursing attendants from 23.25 per cent to 5.2 per cent by the use of face masks. Before the use of masks 8.0 per cent had developed scarlet fever whereas in the three years of their use no scarlet fever had occurred among the nurses.

Doust and Lyon,[3] and Haller and Colwell[4] published simultaneously reports on the relative efficiency of masks made of various cloths and of various sized weaves. This was followed by further work on this same subject by Weaver and Murchie.[5] They showed that sneezing and blowing through the nearly closed mouth produced the heaviest droplet contamination, while coughing with the mouth wide open or talking or whistling resulted in much less droplet contamination. In coughing with the lips only slightly parted there was also heavy contamination. The average range of spread of contagious droplets has been estimated at from eighteen inches by Lynch and Cumming[6] to ten feet by Doust and Lyon, while Weaver and Murchie assume about three feet to be the droplet radius of contagious importance.

From their work it would appear that coughing with the mouth wide open presents less danger so far as droplets are concerned than does the spray-producing explosion resulting from an attempt to smother with closed lips the inevitable cough. In either event we have been taught that we must cover the mouth with the hand when coughing. This brings us to the second means of transmission—the infected hand.

This mode of spread has been emphasized during the influenza epidemic by Lynch and Cumming who ascribed to it even greater importance than droplet infection. Brown, Petroff and Pasquera[7] produced tuberculosis in guinea pigs with the washings from the hands of heavily infected tuberculous patients who had previously coughed onto their hands. They were not able to infect guinea pigs with washings from sterile door knobs which tuberculous patients with demonstrated contaminated hands had handled, or with washings from the hands of individuals who had shaken hands with these patients. On the other hand, Weaver and Murchie showed that at one time or another 35.6 per cent of forty-

five pupil nurses on a diphtheria ward showed hemolyzing streptococci either on the palmar surface of the fingers or under the nails, or in both places. Three per cent of these nurses carried in the same manner diphtheria bacilli. These cultures were made after routine washing of the hands. The same authors cultivated hemolyzing streptococci in 5.8 per cent and diphtheria bacilli in 4.4 per cent of 137 cultures from door knobs in the wards.

Contaminated eating utensils, especially if not properly sterilized, are a potent source of infection. Lynch and Cumming showed that the mess kit rinse water as usually used in the army messes was an excellent spreader of organisms from the utensils of one individual to those of another. Brown, Petroff and Pasquera in a study of the transmission of tubercle bacilli were able to infect guinea pigs with the washings from spoons, forks, glasses, and cups immediately after use by patients with numerous tubercle bacilli in the sputum. Washings from knives and plates did not produce the disease in guinea pigs.

The three modes of conveyance above described have received chief attention but they by no means exhaust the possibilities. Kissing as a means of infection has been studied by the last named authors. They produced tuberculosis in guinea pigs with washings from sterile petri dishes which tuberculous patients had kissed, both immediately and ten minutes after coughing. Dust, particularly the dust of rooms, has been shown to harbor tubercle bacilli, fixed type pneumococci and other organisms, but it has not been conclusively shown that the role played by contaminated dust is of great importance in the spread of infection. This subject requires further investigation. Spread through flies and contaminated food is possible. Calmette[8] states that all cases of tuberculosis which expectorate have tubercle bacilli in the dejecta. The occurrence of organisms causing other respiratory infections in feces has not so far as we know been seriously studied. Maxcy[9] introduced B. prodigiosus into the conjunctival sac and recovered the organism from the stools after twenty-four hours. It may not be venturing too far into the realm of the impossible to suggest that further studies in this field might resurrect the old subject of water-borne infection as in typhoid fever, as one of the means of spread in influenza.

The importance of the eye as a possible portal of entry is emphasized by Maxcy and by Vincent and Lochon.[10] The former introduced B. prodigiosus into the conjunctival sac and recovered it within five minutes from the nose and within fifteen minutes from the throat. The face mask as usually employed, for the prevention of droplet infection does not protect against infection of the throat through the eyes. Maxcy points out that respiratory infections such as acute coryza, measles and influenza begin with conjunctivitis, and that although this symptom in certain cases is simply a suffusion and a part of a general mucous membrane reaction, yet in others the priority and prominence of this symptom indicate rather a primary relationship with the disease. He further states that in the great epidemics of plague that have from time to time swept over the Old World, masking of the entire face, eyes included, has been wonderfully effective.

Most of the experimental work on the use of face masks has been in their ability to prevent the discharge of organisms in droplets from infected individuals, while the application of the principle has been for the protection of those exposed, which is in reality quite another problem. Maxcy has shown that in-

fection with B. prodigiosus can take place through the conjunctival and lacrymal duct even though the mouth and nose be masked. He suggests the use of goggles. Vincent and Lochon recommend the use of a mask which will surround the entire head and which prevents the gauze from touching the face. They found that a thickness of five layers of the gauze used was necessary.

Fate of Organism from the Time It Reaches the Exposed Individual Until Eliminated or Until Invasion Takes Place.—Maxcy reports that the lacrymal secretion is not bactericidal. The sterility of the conjunctival sac is probably maintained by mechanical irrigation through the lacrymal duct. Maxcy recovered in man B. prodigiosus from the nose within five minutes after it had been introduced into the conjunctival sac. Stort[11] found that colon bacilli instilled onto a rabbit's conjunctiva had disappeared within an hour, but that after ligation of the lacrymal duct, the organisms remained present over a much longer period.

Bloomfield, in a study of the mechanism present in the upper air passages for disposing of bacteria, inoculated individuals with pure cultures of sarcina lutea. These organisms applied in large amounts to the tongue, the nasal mucosa or crypts of the tonsils disappeared within a short time, usually within an hour. In one case they were recovered from the nose after twenty-four hours. He enumerates the factors of this protective mechanism in the upper air passages as: (1) mechanical, (flushing action of secretions, with swallowing and ejection of nasal and mouth secretions, and the action of ciliated epithelium; (2) chemical, (including the reaction of mouth and nose secretions with other possible bacterial inhibitory factors); and (3) biologic (including such processes as phagocytosis and the effect of bacteria already present on the invader).

He was unable by cleansing the mouth with one liter of saline to reduce the bacterial content of the mouth secretion below 50 per cent. Fluids with hydrogen ion concentration similar to those of several specimens of saliva had no inhibitory action on the growth of sarcina lutea. This organism did not die out when kept in saline solution or in a mixed growth of normal mouth flora, but did die out, usually within one hour, when it was introduced in varying concentration into saliva. Bloomfield concludes that the rapid disappearance of sarcina lutea from the nose and mouth was due chiefly to the bactericidal effect of the saliva and mouth secretions.

Why is it that with this bactericidal substance in the oral and possibly also the nasal secretions some pathogenic organisms, as for instance the meningococcus, do grow apparently quite freely on the mucous membranes? This subject requires further investigation. Stillman[12] has been able to obtain the influenza bacillus in carriers from the postpharyngeal wall and Sailer[13] and his associates discovered fixed type pneumococcus carriers through the use of nasopharyngeal cultures. Kligler[14] reports that small amounts of nasal secretions added to culture media at times favored the growth of meningococcus organisms. This does not appear to be true for all bacteria.

Interest in streptococcus and diphtheria bacillus carriers has centered chiefly on the bacteriology of the contents of the tonsillar crypts and follicles. Numerous observers have shown that these organisms localize in the tonsillar tissue, and Moss[15] has concluded from experimental evidence that intermittent diph-

theria carriers may show positive cultures at those times when the colonies in the follicles or caseous plugs break forth at the tonsillar surfaces, and become negative when they are entirely within the tonsillar tissue. That this is but one phase of the story is evidenced by failures to cure diphtheria carriers through tonsillectomy.

That the nasal accessory sinuses may serve as reservoirs is shown by the work of various observers. Crowe and Thacker-Neville,[16] found B. influenzæ in 26 per cent of infected maxillary antra during the period of the influenza epidemic and in 21 per cent in a series observed during six years when there was no epidemic.

The Mode of Invasion of the Virus into the Body.—A discussion of this phase in the pathogenesis of respiratory infections will be reserved for a later review.

BIBLIOGRAPHY

[1]Bloomfield, Arthur L.: Bull. Johns Hopkins Hosp., 1919, xxx, 317.
[2]Weaver, George H.: Jour. Infect. Dis., 1919, xxiv, 218.
[3]Doust and Lyon: Jour. Am. Med. Assn., 1918, lxxi, 1216.
[4]Haller, D. A., and Colwell, R. C.: Jour. Am. Med. Assn., 1916, lxxi.
[5]Weaver, G. H., and Murchie, J. T.: Jour. Am. Med. Assn., 1919, lxxiii, 1924.
[6]Lynch, C., and Cumming, J. G.: Mil. Surg., 1918, xliii, 597.
[7]Brown, L., Petroff, S. A., and Pasquera, G.: Jour. Am. Med. Assn., 1919, lxxiii, 1576.
[8]Calmette, A.: Ann. de l'Inst. Pasteur, 1919, xiii, 60.
[9]Maxcy, Kenneth F.: Jour. Am. Med. Assn., 1919, lxxii, 636.
[10]Vincent and Lochon: Bull. l'Acad. Med., 1918, lxxx, 348.
[11]Stort, A. G.: Arch. f. Hyg., 1891, xiii, 395.
[12]Stillman, E. G.: Jour. Exper. Med., 1919, xxxiii.
[13]Sailer et al.: Arch. Int. Med., 1919, xxiv, 600.
[14]Kligler, I. J.: Jour. Exper. Med., 1919, xxx, 3.
[15]Moss, W. L.: Personal communication.
[16]Crowe, S. J., and Thacker-Neville, W. S.: Bull. Johns Hopkins Hosp., 1919, xxx, 322.

—*W. T. V.*

Immunization Against Diphtheria by the Employment of a Toxin-antitoxin Mixture. A Caution

ONE of the most brilliant and most useful discoveries of modern medicine is that which has given us diphtheria antitoxin. This wonderful agent has robbed this distressing disease of much of its horror. I well remember, and indeed shall never forget, the scene in one of the lecture rooms of the University of Budapest in 1894 when Roux read his paper on the employment of this agent. Before that time Von Behring had announced the discovery and published some confirmatory results. Even the Germans present at that meeting were hesitating about accepting Von Behring's claims, but in that mingled audience there was no one who was at all in doubt concerning either the honesty or the skill of Roux. Some years before Roux and Yersin had discovered diphtheria toxin and all assembled that day were ready to accept any statement that Roux might make. He read his paper giving in detail the preparation of the antitoxin and the results which he had obtained in using it in the treatment of diphtheria in children. When he concluded, hats were thrown to the ceiling, grave scientific men arose

to their feet and shouted their applause in all the languages of the civilized world. I had never seen and have never since seen such an ovation displayed by an audience of scientific men. Each of the delegates of that section of the International Congress of Hygiene left Budapest with a bottle of antitoxin in his grip and thus its use was begun in the most distant parts of the earth. Twenty-five years have elapsed since that eventful day at Budapest and it is now conceded that everything claimed for this discovery at that time has been more than justified. Many of us remember when the death rate from diphtheria in many epidemics ran to 70 per cent and even higher. Now we know that when used on the first appearance of the membrane in the throat there are practically no deaths. The neutralization of the toxin by the antitoxin is as sure as that of the neutralization of an alkali by an acid. Hilbert has shown that when the antitoxin is administered late in the first day, the death rate is 2.2. This increases as follows:

On the second day	7.6
On the third day	17.1
On the fourth day	23.8
On the fifth day	33.9
On the sixth day	34.1
After the sixth day	38.2

The value of this agent, however, is even greater than is indicated by the lowering in the death rate. For every sick child saved five are saved from being sick. While the curative value of diphtheria antitoxin is great, its preventive value is greater still. The physician called to a family in which one child has diphtheria gives a curative dose to the sick one and immunizing doses to all others. Even when the sick one has been neglected so long that the curative value of the agent is lost its preventive value is still potent. Diphtheria antitoxin has lowered not only the mortality rate but still more the morbidity rate. Unfortunately however, the immunity produced by diphtheria antitoxin is only temporary, lasting only from three to four weeks. This discovery, as valuable as it is, has not exterminated diphtheria.

Recently a toxin-antitoxin mixture nicely adjusted, so that there is no excess of toxin, has been used for purposes of immunization. The claim is made that permanent immunity against diphtheria is secured by this preparation. It is the purpose of this writing to call attention to the fact that there is danger in the use of this mixture. If it should happen that the toxin is in slight excess lives may be lost. Moreover, the claim that this mixture confers lasting immunity to diphtheria is without scientific justification. No manufacturer possesses the scientific data necessary to justify him in making any such claim for his product. In addition to this there is great responsibility resting upon the manufacturers of this preparation. Should the mixture be improperly balanced and contain an excess of toxin some day some health officer in trying to eradicate diphtheria from his community may find that his zeal has lead him into a most embarrassing situation.

—V. C. V.

Full Time Medical Professors

MUCH has been said in the last few years concerning full time clinical professors in our medical schools. This is a question of considerable importance to the future of medical education in this country. The board of regents and the faculty of the University of Michigan Medical School will make an attempt to demonstrate the value of full time chairs. The regents have decided that hereafter all appointments in this school shall be for full time. There will be no divided allegiance. From the head of the department down to the humblest assistant all shall give full time to university work. The clinical professors will be paid two salaries, one as a teaching professor and the other as a clinician. The first salary will be equal to the salaries paid men of equal rank in the other departments of the University and the second salary will be paid from the hospital funds. This school now has a hospital of over 400 beds, but these fail to accommodate all the patients who come to Ann Arbor. The legislature has authorized the board of regents to build and equip a new hospital which will furnish 800 additional beds. This should give ample accommodation for a time at least. The hospital is a part of the University and a state institution. The state makes special appropriations for the hospital and it seems willing to pay the men who serve in this institution in taking care of the sick and injured people in the state. The hospital salary will be determined by the skill and ability of the individual filling the position. In other departments of the University the teacher has but one market value and that is as a teacher. On the other hand the clinician has two market values, one as a teacher and one as a clinician. This fact seems to justify the action taken by the board of regents. It must be quite evident that the full time plan will not work when it is not applicable to all members of the teaching and clinical staffs. In the new University of Michigan hospital, as in the old one, there will be neither free beds nor beds set aside for the financial support of members of the staff. Every patient is used or may be used for teaching purposes. It has been demonstrated that this may be done when good judgment is exercised not only without injury to the patient but actually to his benefit. Of course it will be understood that the University can not pay to any of its professors the great sums now being earned by many men in private practice. But the University intends to pay a liberal salary to its clinical men and it is believed that good men will be found on these terms. They will not have extravagant incomes but they will have enough to live upon decently, support their families in comfort, attend medical societies, go abroad and keep up with the profession. In addition they will have opportunity to do consecutive scientific work, to contribute to the advancement of their specialties and to make for themselves reputations both as teachers and as contributors to scientific knowledge. While there may be still some doubt as to the success of this plan there can be no question that part time clinical instructors do not give satisfactory results. The man whose time is divided between earning a living and seeking the comforts and even the extravagances of his family, and his duty as a teacher is almost certain to be influenced most largely by the former. There has been a marked evolution of the medical school in this country. Less than a century ago a group of ambitious medical men in any city could charter a

medical school, bring in students and lecture or hold clinics whenever they were not engaged in private practice. This resulted in a large number of medical schools giving poor instruction and failing to adequately instruct students. The members of such faculties as a rule did not expect to make money directly out of their schools but they did seek to enhance their reputations and expected their graduates would call upon them when in need of consultation. Later it became the custom to pay the men in the medical schools who were engaged in laboratory and more strictly scientific work. This has resulted in great advance in teaching the fundamental sciences in our medical schools. So great has been this advance that it is probably no exaggeration to say that our best medical schools today are giving the scientific fundamentals as well as they are taught in any part of the world. To do the same for the clinical branches it will be necessary to secure the full time of those who instruct in these subjects. The outcome of this move in the department of medicine and surgery of the University of Michigan will be watched by the profession with interest.

—*V. C. V.*

Medical Veterans of the World War

UNDER the above name there was organized at the last meeting of the American Medical Association, June, 1919, a society for which a constitution and by-laws were adopted and officers for the year elected. Those eligible to membership include the following groups:

1. All medical officers, contract surgeons of the United States Army and Act. assistant surgeons of the United States Public Health Service who have served in the medical corps of the United States Army, United States Navy and the United States Public Health Service.

2. All medical members and medical examiners of local medical advisory and district boards, officially appointed by the President of the United States, the Provost Marshall General of the United States Army and the Governors of the various states.

3. Members of the medical profession of allied nations who have been in the service of their governments during the world war shall be eligible to associate membership.

The officers are a president, vice-president at large, one vice-president for each state and territory and a secretary-treasurer. The vice-president for each state with two other members from such state shall constitute a state council.

Each member shall pay one dollar to the treasurer as annual dues. The treasurer is Colonel F. F. Russell, M.C., U.S.A., Army Medical School, Washington, D. C., to whom application for membership should be made.

It is hoped that a chapter will be formed in each state. The next meeting will be held in New Orleans, La., at the time of the annual meeting of the American Medical Association. The present membership of this society is about 2200. It is estimated that there are about 50,000 men eligible to membership.

The objects of the association must be self-evident. In the first place it is desirable to have at least annual meetings when the good comradeship begun in

camp or field may be renewed. In the second place it is desirable to perpetuate the memory of the hundreds of the profession who gave their lives in the great conflict. And finally those who fought to make the world safe for democracy should have some interest in determining what kind of democracy we are to enjoy. It is not the intention of the founders of this society that it should become a political organization but it is expected that this association will interest itself intelligently and unitedly in every policy which promises good to the nation as a whole and will oppose every move to the contrary. If the world is to advance in the right direction the medical profession must aid in such advancement. Individually the medical man has but little influence but collectively he can speak with a voice which will be heard even to the most remote parts of the country. Nearly one half or more of the legally qualified physicians of this country of military age responded to their country's call in time of need. No other profession or group of men made such a record. In the hospitals in this country and in France the sick and wounded American soldier was ministered to by men for the most part unaccustomed to war and only recently removed from civil practice. To this duty the American physician gave the best that was in him. Whether we remain in peace or be again involved in war the medical profession must prove a mighty factor in the evolution of the race. It is to be hoped that the muster roll of the Medical Veterans of the World War will become longer and longer until it includes every eligible name.

—*V. C. V.*

The Required Year of Hospital Work

SOME years ago certain medical schools, with best intentions, decided to require a hospital year of all their students before graduation. This example has been followed by other schools and an attempt has been made to have all Class A medical schools adopt this requirement. Is this wise? No medical school in this country is today prepared to guarantee to all its students who finish their fourth year satisfactory hospital appointments. At best the medical school controls only a limited number of appointments as interns in its own hospital or in hospitals dominated by members of the faculty. It follows from this that the faculty can not supervise, properly direct or in any thorough way control the hospital instruction which its students receive. It must therefore give credit for work over which it has had no direction. Is this wise? It should furthermore be borne in mind that all those who go to medical schools are not to become practitioners of medicine. Some, and it is to be hoped that among these will be found the brightest students in the class, have no intention of practicing medicine; they are going into medicine in order to do scientific work. I am aware of the fact that it has been proposed that these students should be provided for by allowing a year of laboratory work to count for an equal time in the hospital. But it has happened in at least one state that when the legislature was asked to pass a law requiring a year of hospital work before graduation in medicine, the law makers failed to make any provision for the substitution of laboratory for hospital work. You can hardly expect a young man who intends to go into pathology or physiology to delay his work along these lines by taking a hospital year; prob-

ably in some hospital where he serves only as an orderly and the highest type of scientific work that he is called upon to do is to test samples of urine for albumin or sugar or occasionally to examine microscopically a bit of tissue or more rarely to make an autopsy. The result of requiring a hospital year of medical students before graduation is going to be most disastrous to the fundamental scientific branches of medicine. It will be found more and more difficult to fill the laboratory positions with competent medical men. From this one of two things must follow. First, the scientific work in our medical schools will be done more and more by nonmedical men. Students who intend to work in physiology and pathology will not graduate in medicine because to do so would compel them to spend a year in hospital work, most likely under conditions which would be unpleasant and by no means helpful. The second alternative is that the scientific work will be done by medical graduates who have been compelled to take the hospital year and in doing so have acquired a dislike for medical practice. This is a serious matter and already the heads of laboratory departments are complaining because the brightest students are lured into clinical medicine or surgery. There is still a bigger problem, one which possibly may influence greatly the future of the profession. The demand for full time health officers is growing day by day and it is not beyond the range of possibility that within twenty-five or fifty years every city of twenty-five thousand or more inhabitants and every densely populated county will demand full time health officers. There is nothing in the ordinary routine of a hospital year which in any way fits a man for the duties of a health officer. He gets in the hospital no instruction in epidemiology and but little in the control of infectious diseases. He learns only the rudiments of the influence of diet on health and the effects of various foods upon disease remain unknown to him. In view of all these things I suggest that the medical schools that have not already required a hospital year before graduation of their students should stop and think.

The question of a hospital year before admission to the practice of medicine is another question. There is no doubt about the wisdom of this requirement. Therefore the only conclusion is that the requirement of a hospital year in medicine should lie with state boards of medical examiners and not with medical faculties. —*V. C. V.*

*A New Book on Pellagra**

NO one, in this country at least, is more competent to write upon this subject than the author of the volume which now lies before us, Dr. H. F. Harris, of Atlanta, Ga. Dr. Harris has made a most important contribution to the literature of this interesting disease. He has given us a most faithful and accurate résumé of the work done on this subject, especially that of Italian physicians. As is well known the author is a firm believer in the theory that this disease is caused by maize or Indian corn. However, he modifies the maize theory inasmuch as he believes that Indian corn must be eaten through two or more generations before it develops the disease. His conclusions are so well stated by himself that we herewith reproduce them as follows:

*Pellagra, by H. F. Harris, M.D., The Macmillan Co., 1919.

"Pellagra is an extremely chronic affection of temperate and subtropical countries, i. e., where Indian corn is grown and much eaten. While the malady has been generally thought in the past to be the consequence of inanition, the result of an adequate diet, it has been more recently regarded as the effect of the habitual consumption of Indian corn, and possibly in rare instances of eating other starchy foods that have been acted upon by low vegetable forms. If this theory should be found to be correct the disease is probably more directly the result of the action of certain phenol poisons, produced by moulds while growing in these cereals, and possibly of albuminous and ferment toxins contained in sound maize, all of which together, acting from one generation to another, and not unlikely intensified by bad hygienic conditions and insufficient and imperfect food, ultimately culminate in a frank outbreak of the classical symptoms of this disease. Finally, it can not be too strongly urged that the malady is probably always hereditary, no person ever in his lifetime eating enough maize to produce the disease."

The author rejects all theories founded upon the belief that the disease is of bacterial origin or is contagious. In this he is undoubtedly right. The transmission of pellagra to the lower animals, to apes, and to man, has been tried in every conceivable manner and has always ended in failure. Certainly we are justified in concluding that pellagra is not an infectious disease.

The half baked and visionary theory of Sambon that pellagra is transmitted by the bite of a gnat receives the ridicule which it deserves. Sambon seems to have been a Dr. Cook who hurried to Italy to announce a discovery which the best Italian physicians had been seeking for centuries. The distinguished Roumanian bacteriologist, Babes, expresses himself concerning Sambon and his announced discovery as follows: "An English Commission, which had probably never seen pellagra, on arriving in a region where the disease was common, and after seeing the pellagrins, and observing that there was a small gnat which produced a redness on the skin immediately after biting, promptly telegraphed to the world that the insect was the cause of pellagra."

We must admit that however much we have enjoyed and profited by Dr. Harris' new book, we fail to see where he has done justice to Goldberger and his associates. The author scarcely mentions the work of Goldberger. Having seen the results that have followed the change in diet as recommended by Goldberger, especially as demonstrated in the large asylum at Milledgeville, Ga., we think that Goldberger's conclusions deserve more consideration than is shown them by Dr. Harris. However, no one interested in this disease should fail to read the book by Doctor Harris.

—*V. C. V.*

The Pathogenesis of Pulmonary Tuberculosis

OUR ability to distinguish human and bovine strains of the tubercle bacillus has been of assistance in studies of the mode of invasion of those organisms into the body. It is now quite generally accepted that about 10 per cent of cases of tuberculosis in humans is due to the bovine tubercle bacillus, and that in all probability this type of infection, coming from cows' milk, passes through the tissues of the alimentary tract as a portal of entry. Infection of this

type usually occurs in infancy or childhood, adults being relatively immune to the bovine organism.

Concerning the primary site of the thoracic lesion in pulmonary phthisis, there is still difference of opinion. One theory is that the lesion is usually primary in the lymph glands, particularly the glands around the hilus of the lungs, and that involvement of the lung parenchyma follows spread from these primary foci usually along the bronchi or the peribronchial tissues. The opposite tenet is held by some,—that the glandular involvement is usually or always secondary to invasion of the lung parenchyma. Ghon believes that the focus is primary in the lung and that at necropsy it is found to be small, varying in size from pinhead dimensions to that of a cherry, being most often about the size of a pea. It is often smaller than the "secondary" focus in the glands, the glandular material being particularly prone to hyperplastic changes, but Ghon explains that the apparent age of a lung focus is always as great or greater than that of the glandular process. It is chiefly on this pathologic distinction that he bases his theory of parenchymatous priority.

Ghon states that the mediastinal glands which one finds to be tuberculous are those that would become so from a primary focus in the lungs, and that on following out the drainage area from the involved glands into the lung tissue, a tuberculous lesion is almost always found in the latter area. The changes in the glandular structure never appear to be older than do those in the parenchyma.

The primary lung focus is described as usually single (72 per cent), rarely occurring as more than two or three lesions, usually situated immediately beneath the pleura which frequently becomes involved, and rarely located at the apices. It may occur in any lobe, being present in 31 per cent in the right upper lobe, in 23 per cent in the left upper lobe, in 22.5 per cent in the right lower and least frequently in the right middle lobe. The commonest site is in the anterior surface of the upper lobes, midway between apex and interlobar fissure. In the lower lobes the most frequent site is near the lower edge posteriorly.

Lymphatic drainage from these areas passes through the bronchopulmonary glands to the tracheobronchial group. The inferior tracheobronchial, those beneath the angle made by the right and left bronchi, receive the greater portion of the drainage from the lower portions of the lungs, while the right and left superior tracheobronchial systems, located in the angles between the bronchi and the trachea, receive from the upper portions. Ghon shows that the diseased glands lie in the drainage system collecting from the regions of the primary lung foci. Numerous anastomoses connecting the various sets of glands explain, according to him, apparent deviations from this rule.

Canti reaches conclusions similar to those described by Ghon. In sixteen cases studied, single lung foci were discovered in eight, and multiple in two. Among the remaining six no "lung foci" as described were found, but abdominal tuberculosis was present in all. Tuberculosis of the mediastinal glands, other than in acute miliary tuberculosis, was found in twelve of the sixteen cases. In ten the glands were located in anatomic relationship with a "lung focus" and in two no focus could be found. In these latter there was extensive accompanying abdominal tuberculosis.

The lung focus was usually caseous or fibrocaseous and averaged the size of a pea. One was calcareous, one was liquefying and one showed cavitation. The

most severely infected mediastinal glands were recorded as being in caseous or fibrocaseous state.

Canti, in claiming that the lung focus when present is the original tuberculous lesion in the body, emphasizes the facts that the focus is usually single, that when it is present there is in the majority of cases no other lesion in other organs indicating another portal of entry, that when the portal of entry appears to be elsewhere, as in the intestines, there is usually no lung focus, and that there is a quite constant relationship between the occurrence of lung foci and tuberculous mediastinal glands.

The work of Ghon and Canti does not prove conclusively the priority of lung infection, but it does serve to remind us that the mode and site of invasion of the tubercle bacillus in pulmonary tuberculosis is still a debated question. It remains to be proved that in two such different tissue structures as lung parenchyma and glandular tissue a caseous or fibrocaseous process in the latter is necessarily of more recent duration than a calcified or cavitated lesion in the former. Ghon found only one case with lung focus which showed no involvement of the mediastinal glands, while there were seven showing diseased mediastinal glands without lung foci and with no evidence of a source of infection outside the lung. This appears somewhat contradictory to the theory.

BIBLIOGRAPHY

Ghon, A.: The Primary Lung Focus of Tuberculosis in Children, trans. by D. Barty King, Lond., 1918.

Canti, R. G.: Primary Pulmonary Tuberculosis in Children, Quart. Jour. Med., 1919, xiii, 71.

—*W. T. V.*

Dr. Elmer Ernest Southard

It is with the deepest sorrow that we record the death of one of the most brilliant members of our editorial staff. Dr. E. E. Southard was born in Boston, July 28, 1876. He took his A.B., A.M., and M.D. degrees from Harvard University, taking the last mentioned in 1902. Following his graduation in medicine he studied in Germany. In 1906 he married Dr. Mabel Fletcher Austin, of Boston.

He became successively Assistant, Instructor and Professor of Neuropathology in his Alma Mater. From 1906 to 1909 he was pathologist to the Danvers State Hospital. From 1912 to his death he was Director of the Boston Psychopathic Hospital. He was a member of the American Academy of Arts and Sciences, the Association of American Physicians, the American Medical Association, and the American Association of Psychologists.

Dr. Southard was a writer of forcible English and was thoroughly sound in all his conclusions. A few weeks before his death he completed a compendious work on Shell Shock. With Dr. Gay he contributed very largely to our knowledge of anaphylaxis. Readers of this journal have profited by some of his best writings. He died in New York City February 8, from influenza-pneumonia within forty-eight hours after he was taken ill. He was one of the most brilliant contributors in this country to his specialty.

We extend to his wife and children our deepest sympathy. The medical profession of this country has lost in early life one of its most brilliant and productive workers.

—*V. C. V.*